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A COMPARATIVE STUDY OF CONIDIAL FORMATION IN CEPHALOSPORIUM AND SOME RELATED HYPHOMYCETES[1]

M. ELIZABETH PINKERTON

p. 89: under *H. pinicola* read—Pl. 12, figs. 72 a-l, 73 a-e.
p. 92: under *H. caroliniana* read—Pl. 13, figs. 76 a-e.
p. 97: first sentence, second paragraph, insert *no*—From the table following it is clear that there is *no* . . .
p. 124: the explanation of plate 12 should include figs. 72 and 73, now on p. 126.

solution. Upon [illegible]
growth was homogenous and scanty on the surface and cloudy-white within the medium. Microscopic examination revealed a pseudomycelium bearing numerous lateral spore balls reminiscent of *Cephalosporium* (pl. 2, fig. 43). This suggested a further study of the cause of the deviation and the relationship of the budding and cephalosporial states. A cursory investigation of the pertinent literature disclosed much confusion regarding the types of Hyphomycetes which form cephalosporia (spore balls). Hence, the problem has been extended to a comparative morphological study involving principally

[1] An investigation carried out in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

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A COMPARATIVE STUDY OF CONIDIAL FORMATION IN CEPHALOSPORIUM AND SOME RELATED HYPHOMYCETES[1]

M. ELIZABETH PINKERTON
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I. Introduction

Among the organisms isolated by the author from dermatomycoses at the Barnard Free Skin and Cancer Hospital, St. Louis, Missouri, a red yeast, identified as *Torulopsis mucilaginosa,* appeared frequently. On ordinary media it formed a rose-red, pasty, and superficial colony; in liquid, a basal deposit and some pseudomycelial proliferation throughout the solution. Upon nitrate agar of Stellung-Dekker ('31) the growth was homogenous and scanty on the surface and cloudy-white within the medium. Microscopic examination revealed a pseudomycelium bearing numerous lateral spore balls reminiscent of *Cephalosporium* (pl. 2, fig. 43). This suggested a further study of the cause of the deviation and the relationship of the budding and cephalosporial states. A cursory investigation of the pertinent literature disclosed much confusion regarding the types of Hyphomycetes which form cephalosporia (spore balls). Hence, the problem has been extended to a comparative morphological study involving principally

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forms which may grow parasitically or saprophytically upon the human skin.

It is the primary aim of this dissertation to explain the mechanism of cephalosporial production and to correlate such findings with data on other types of spore-producing organs. Observations of the pathogenicity of these organisms for man are recorded. Also, on the basis of methods of production and kinds of spores, as well as upon other physiological and morphological observations, the group is discussed systematically. Present treatments are criticized and emended, at least in consideration of the few forms studied.

II. Materials and Methods

Investigations have been made upon the following species. For further discussion of systematics see p. 37.

ORGANISM	PLACE OBTAINED
Allescheria Boydii Shear	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium kiliense (Gruetz) Hartmann	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium nivcolanosum Benedek	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium rubrobrunneum Nannizzi	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium Serrae Maffei	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium Stuchmeri Schmidt & Beyma	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium symbioticum Pinkerton	Barnard Free Skin and Cancer Hospital, St. Louis
Clonostachys Araucaria Corda	Centraalbureau voor Schimmelcultures, Baarn
Clonostachys Araucaria var. *rosea* Preuss	Centraalbureau voor Schimmelcultures, Baarn
Clonostachys Araucaria var. *confusa* Pinkerton	C. Thom
Clonostachys spectabilis (Harz) Oudem. & Sacc.	Centraalbureau voor Schimmelcultures, Baarn
Gliocladium deliquescens Sopp	C. Thom
Gliocladium fimbriatum Gilman & Abbott	C. Thom
Gliocladium penicilloides Corda	C. Thom
Gliocladium Vermoeseni (Biourge) Thom	C. Thom
Scopulariopsis brevicaulis (Sacc.) Bainier	C. Thom
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli	Barnard Free Skin and Cancer Hospital, St. Louis

The stock medium was glycerine agar (nutrient agar plus 6 per cent glycerine, pH 6.9). The following agars were also used: maltose (pH 5.4), Sabouraud's dextrose (pH 5.6), wort (pH 4.8), Raulin's (pH 4.15), Czapek's (pH 4.43), Richards' (pH 4.36), Endo's (pH 7.52), Gorodkova-Maneval modified (pH 6.8), nitrate (pH 5.5), nutrient (pH 6.0), corn meal (pH 6.0), potato dextrose (pH 5.7); these liquids—nutrient (pH 6.0), lactose (pH 6.8), litmus milk; also sterilized carrot plugs, gypsum blocks, Bacto-beef blood serum (pH 7.3), and glycerine agar double strength plus 50 per cent Bacto-blood (pH 6.0). The latter gave the most luxuriant and rapid growth.

Cultures were made chiefly in test-tubes and bits of them were examined at intervals in semi-permanent mounts of Amann's ('96) lacto-phenol plus a 1 per cent aqueous solution of anilin blue. Such served for gross morphology and even showed a fair degree of the more minute structure of the phialide. The proper relationships of the various parts of the organism and their comparative colonial characteristics are shown in the photographs of Petri plate cultures containing Czapek's agar (pl. 6). Cultures were also examined directly under high power after placing a cover slip over a sparsely grown section of the agar. Camera-lucida sketches were made of relevant structures and photomicrographs were taken where possible, but due to the minuteness of the conidial fructifications the latter procedure was not very practical.

Hanging drop and agar preparations made in van Tieghem cells were of little use, since the fruiting bodies were produced pendant in the air and hence not discernible under oil. Agar slide cultures, produced by placing a few drops of warm agar upon a sterile slide, inoculating, and applying an aseptic cover slip (No. 1, 24 × 40) when the agar was cool enough to barely spread, were found to be most helpful, giving a comparatively small circular agar matrix from which aerial fructifications radiated. The narrow ends of the cover slip were sealed with paraffin to make the mount rigid, and the microcultures incubated at room temperatures in sterile staining jars with moist filter-paper at the bottom. On the second day the formation of spores could sometimes be observed under oil immersion.

Material for cytological study was grown on Raulin's agar and on glycerine agar. Hermann's fluid[1] was employed for killing and Jeffrey's celloidin technique was followed for the earlier work. The fixing agent was applied directly to the slant culture which was evacuated for about fifteen minutes to remove air from the agar containing submerged growth. After twelve hours the material was washed for an equal time in slowly running water, following which the agar was cut into small blocks about 5 mm. square and run through the alcohols.

Since it was impossible to get celloidin sections thinner than 10 μ, for studying structures of 2 μ average diameter the paraffin method was tried. After the agar culture had been cut into small bits, good infiltration was secured and sections were obtained 1, 3, 5, 8, and 10 μ in thickness. The very thin ribbons had the disadvantage that so few aerial phialides were encountered in a slice. Nevertheless, nuclear orientation was clearer in the paraffin material, whereas accessory cytoplasmic inclusions and external mucoid structure were more noticeable in the celloidin matrix. Haidenhain's iron-alum haematoxylin was used exclusively for staining, applied according to the following schedule: hydration, one hour in 20 per cent USP hydrogen peroxide, washing, 4 per cent iron alum two hours, ½ per cent haematoxylin twelve hours, slight destaining with 2 per cent iron-alum while watching through the microscope, dehydration, clearing in xylol, and mounting in Canada balsam. The species cytologically investigated include: *Allescheria Boydii, Clonostachys Araucaria, C. Araucaria confusa, Cephalosporium Serrae, Gliocladium deliquescens,* and *G. penicilloides.*

An attempt was made to compare the wall composition of the ordinary mycelium and of the phialide by use of the polarizing microscope. The thinness of the cell walls and the difficulty of obtaining sufficient light with the higher magnifications prevented satisfactory observation. Hanging drop cultures, where the fructifications could be brought into focus, served best.

[1] The formula is: platinic chloride 3 cc. in 12 cc. of distilled water; osmic acid 2 cc.; glacial acetic acid 1 cc. A fresh solution should be used for each application.

All of the organisms were grown at 30° C. The cultures were usually incubated in the light, since darkness often caused loss of color without other apparent changes. In an attempt to induce the perfect stage, glycerine agar was employed with H-ion concentrations from 3.8 to 7.6; and the following special media were utilized: gypsum blocks, Gorodkova-Maneval medium, carrot plugs, and corn meal agar. Attempts at cross fertilization were made, and while no perfect states were induced some interesting subsidiary observations were obtained.

III. The Conidial Apparatus

A. THE CONIDIOPHORE

The forms here investigated are believed to be closely related to *Penicillium* of the sub-family Aspergilleae in the Hyphomycetes. The form-genus finds its perfect stage in *Carpenteles* Langeron ('22) of the ascomycetous order Plectascales, although the latter name is by no means in common usage. Its conidial apparatus consists of a basal stalk cell from which branches arise variously but more or less regularly for each species. The end cell of the penicillus, called the phialide, cuts off spores terminally in chains. The spores are temporarily adherent, due to connectives which later become disjunctors and probably are formed from the old wall of the phialide. In the material of the present study there are no disjunctors, and the spores mutually adhere in a more or less globular mucilaginous mass around the phialide. The spore ball is termed the cephalosporium in contrast to the entire fructification, or cephalophore. In most cases the cephalosporia are characteristic of the aerial mycelia, although submerged ones have been noted in *Cephalosporium Serrae*. The complete conidiophore varies from a short branch no longer than the spores produced (e. g. *Allescheria Boydii*) to a complex structure equivalent to the penicillus (e. g. *Gliocladium*); but in all cases where cephalosporia are produced the mechanism seems identical.

1. HISTORICAL

a. On conidial production in the Aspergilleae and related forms.—Brefeld ('74) was one of the earliest to observe criti-

cally conidiophore formation although his methods were necessarily crude. He saw no need for the assumption of a special membrane outside that of the conidium, and thought that the process was one of budding. Referring to the forms with disjunctors, Seynes ('86) stated that the phialide wall formed the connective and the conidia differentiated their own walls within. The following statement of de Bary's ('84) exhibited keen insight although his interpretation was incorrect:

> Bei reichlich inner halb enger und mit enger Mündung versehener Behälter abgeschnürten Sporen wird durch die Gallert- oder Gummiabscheidung die Entleerung aus dem Behälter bewirkt, indem die durch Wasseraufnahme quellende Masse aus der Mündung hervortritt. [p. 75].

He believed that there was a gelatinous substance around the spore (a product of its outer lamella) which became deliquescent by addition of moisture and so caused coherence.

A number of investigators attempted to interpret the method of spore production in *Thielavia basicola* of the Plectascales, following Zopf's original description in 1890. He reported an acropetal succession of spores whose lateral walls were differentiated into two layers, the outer being a sheath through which the conidia emerged successively due to the swelling of the mucilaginous middle lamella upon the addition of water. Gilbert ('09) thought that endoconidia were formed in the phialides and emitted consecutively by the force of the growing protoplasm in the basal part of the cell. Brierley ('15) was the first to make a definite attempt to determine the method of conidium production, and he concluded that the method previously called endoconidial was in reality merely acrogenous, being peculiar only in the mechanism of liberation. The conidium put down an additional wall within the parental one which was then ruptured and the new spores were constantly formed from below. The illustrations of the mode of new wall differentiation are quite anomalous. It was Duggar's view ('09) that the endoconidia were ejected by osmotic pressure. Guegen ('05) cited *Gliomastix chartarum* as an example of endoconidial production.

Buchanan ('11) thought that the spores of the cephalosporium were formed by the abstriction of the tip of the

sporophore, the amount of moisture in the air apparently conditioning the amount of adherence. He regarded as unnecessary the separation of *Hyalopus* and *Cephalosporium* upon the basis of more slime in the former. Studies of *Cephalosporium* spp. on insects led Petch ('28) to agree with Buchanan that the spores were really cut off in succession but became aggregated by the secreted mucilage. In a damp atmosphere some were found in loose chains due to the dissolution of the mucilage. He too disregarded the separation of *Hyalopus* and *Cephalosporium.*

In *Penicillium digitatum* Scaramella ('28) illustrated the rupture of the parent sheath anew below each successive spore. Thom ('10) thought that the spores were merely acrogenous disjunctions which produced their own internal walls and were held together by the phialide wall which did not necessarily rupture until late. This would seem to indicate continued elasticity of the conidiophore wall below the tip. In 1930 he added the following hypothesis regarding submerged cephalosporia:

> . . . that the impulse to produce conidia when acting in a submerged hypha in which all parts are in constant contact with the nutrient, produces a transformed branch bearing spores which tend to enlarge, round up as if for germination, and to lose the catenulate arrangement. This may produce a very peculiar spore attached as a single terminal cell or a cluster of cells not recognizable as conidia and without definite arrangement around the tip of the sterigmatic cell. [p. 73].

Wakyama ('31), in cytological investigations upon *Aspergillus* spp., indicated that the daughter nucleus migrated through a sterigma very similar to the condition in the basidiomycetes, but he did not further investigate the mechanism. Finally Dodge ('35) suggested that the mechanism of conidium formation in the genus *Cephalosporium* is probably close to that of *Aspergillus* and *Penicillium* although the conidiophore is not flask-shaped. If the spores are essentially in chains, they slip past one another very soon to form spore balls.

b. Previous interpretations of the mechanics of spore production in general.—There are probably more common factors in all types of spore production than is generally realized, be-

cause the cutting out of similar small masses of protoplasm would not have many physical possibilities of variation. There have been numerous papers regarding this process in the sporangia of the Phycomycetes, a few on ascospore delimitation, and a number on the basidiospore release; whereas, except for Brierley's work, the conidia have generally been neglected.

There are a number of forces which have been championed for spore formation: vacuolation associated with exudation of water or some other liquid; centrosomes or similar bodies which are centers of force and direct the action of protoplasmic streaming; surface tension; Golgi apparatus or the like associated with secretion; and electric forces.

VACUOLES AND EXUDATIONS.—Harper ('99) saw in the sporangium of *Synchytrium decipiens* an early shrinking of protoplasm probably associated with a throwing off of water which accumulated in vacuoles wherein cleavage furrows arose simultaneously throughout the sporangium. In certain cases uninucleate masses were separated, in others multinucleate. He explained the inclusion of a nucleus by the characteristic chemical contents of nucleo-proteins and nucleic acids which were centers of moisture retention or at least had a greater affinity for water than the surrounding cytoplasm.

DeBary ('84) attributed the bursting of the ascus to increased vacuolization due to the absorption of water. In the cutting out of the sporangiospore segments of the Phycomycetes, he mentioned delimiting granular plates which somehow became gelified.

Exuded droplets were shown by Buller ('22) to be significant in the disjunction of basidiospores in all Basidiomycetes except the Gasteromycetes and the Ustilaginaceae. He found droplets also associated with the conidia of the Hymenomycetes and with the aeciospores of the rusts, but thought that such were merely passive. It is possible in these cases too, that, although not effecting violent spore disjunction, exudates may somehow bring about their release. In *Coprinus sterquilinus* he noted that a drop of water 5 μ in diameter appeared at the base of the basidiospore within ten seconds before its abjunction, and that

the liquid was shot off with the spore and doubtless aided in attaching it to the substratum. It was supposed that the liquid came from the hilum, and was largely water, rather than local mucilagination from the sterigmata (often so narrow as 0.5 μ). The latter, however, must have been ruptured, for it soon collapsed. Buller regarded the production of similar drops in *Sporobolomyces* as suggestive evidence that the genus belonged to the Basidiomycetes in spite of the reduced vegetative growth and completely uninucleate state (Guilliermond, '27). However, Stempell ('35) believed that the water-drop mechanism was merely an ecological response and not of phylogenetic importance. He reported its occurrence in association with both the sporidia and secondary conidia of *Entyloma* of the Tilletiaceae, and also with the sprout cells of *Taphrina*, usually included in the Ascomycetes.

Buller thinks that the hydrostatic pressure of the basidial vacuole is very important in forcing the protoplasm and nucleus through the narrow sterigmata and in the final spore discharge, since the basidium collapsed immediately after the final release of one set of spores: "Now my experience, based upon extensive observations on the living hymenium, is that a basidium never produces more than one generation of spores and that after producing a single crop of spores, its sterigmata and body quickly collapse." [Buller, *l. c.* **2**: 28.]

Centrosomes, Surface Tension, and Golgi Apparatus.—Maire's ('02) illustration of basidial development in *Psathyrella disseminata* first suggested a centrosomal interpretation of the terminal granules in the imperfects studied. The four centrosomes in the former, produced simultaneously with the four nuclei, situated themselves at the apex and followed the protruding sterigmata with tails of streaming protoplasm ("cordons kinoplasmiques") eventually drawing the nuclei into the spores. These structures, formerly believed to be confined to fungi, algae, and animal cells, are now being discovered frequently in the higher plants. They are apparently centers of energy associated with the action of the spindle in the separation of chromosomes, as well as with the movement

of flagella and cilia, and are generally believed to be absent in the resting cells. There has been controversy over their origin —cytoplasmic or nuclear—and confusion of them with other structures.

Faull ('04) concluded that the centrosomes were of extranuclear origin and occurred after reforming of the membrane. Guilliermond, Mangenot, and Plantefol ('33) stated that the centrosome was still an enigma and if it aided in ascospore delimitation, its influence was purely physical, perhaps by the superficial tension on the surface of the asteral parasol which conditioned the formation of new walls. Harper ('99) firmly believed that the aster rays (fibrous kinoplasmic elements) cut out the ascospores and formed the limiting walls. They might well direct the spore delimitation but were themselves included within a special wall, according to Faull ('04). Surface tension and perhaps a different chemical composition kept the endospores discrete from the epiplasm.

Buller ('09), in discussing surface tension and the physics of the jet theory of the ascus, stated that a free cylinder of any liquid under the influence of surface tension became unstable as soon as the length was greater than πD. It is a necessary consequence that if once realized the diameter of such a cylinder will divide into as many equidistant spore lengths as πD is contained in one length. Some such physical interpretation must be necessary for the regulation of the size of the endoconidial segments in the forms studied by the author.

Scarth ('27) thought that a definite structural basis of protoplasm was necessary for life movements. He observed a reciprocity of substance in the increase of mitochondrial elements correlated with the disappearance of the active kinoplasm as a result of vital staining; and concluded that the characteristic component of the chondriome, viz. lecithin, entered into the composition of the kinoplasm, and that the mitochondria merely served as reserve substances for the formation of the more labile elements. Vacuoles were thought to arise from the kinoplasm by the rounding up of fibrillae or as blisters or bubbles from the covering films of the chloroplasts or of the central

vacuole. The wandering streams of protoplasm in the cytoplasm condensed into mucilaginous-looking globules which distended as vacuoles; and an essentially similar process effected the cleavage of coenocytic sporangia. In some cases, however, fissuring arose from fibers that radiated from a central body attached to the nucleus, in others by invaginations of the ectoplasm or by outgrowth of vacuoles. The limiting film of these extensions of the kinoplasm became the plasma membrane of the spores, while the fluid which was secreted between them was presumably analogous to the sap which formed in the vacuole.

Sharp ('34) regarded the divisions by constriction, seen in unicellular algae, in the budding of yeast cells, and in the abstriction of conidia and basidiospores, as special cases of cytokinesis by furrowing. The centriole, or inside of the centrosome, has been generally believed to be a point of high surface tension, and the aster, according to Sharp, might be primarily an expression of streaming movements in the cytoplasm which is somewhat more solid than the rest of the more gelified granular protoplasm. He further said that in the plant cell the furrows probably separated regions of relatively high viscosity even though no aster, in the ordinary sense, was present: "Alterations in surface tension and viscosity, together with protoplasmic streaming, are obviously important factors in cytokinesis of certain types, but comparatively little is known about the initial causes of these phenomena."

Wilson ('25) mentioned the appearance in epithelial muscular and glandular cells of a chromidial apparatus assumed to be extruded from the nucleus and of particular function in trophic actions of the cell. He also thought that there was a definite karyoplasmic ratio which regulated cleavage to produce a fixed or typical cell size at a given stage rather than a fixed number of cells. It was the surface of the nucleus and not its volume that was directly proportional to the number of chromosomes.

The Golgi apparatus is known only in animal cells and is connected with secretion. In goblet cells of the colon, secretory

granules have been said to have originated in mitochondrial (chondriosomal) regions and to have migrated to the Golgi region where they were transformed into mucin granules (Duthie, '33). The granules were then liberated into the cytoplasm, finally dissolved, and passed as liquid secretion into the lumen. This apparatus has also been suggested as a center for the formation of enzymes. Whether a similar process occurs in plants with liberation of mucin has not been investigated. The function and limitations of mitochondria are also disputed questions. Guilliermond ('29) homologized the plant vacuome —which he thought arose *de novo* from the cytoplasm—with the Golgi apparatus and Holmgren canals of animals. Nassonov ('23) believed, on the basis of staining experiments, that the vacuolar membrane had a secretory function and was homologous to the Golgi apparatus.

Weier ('32) thought that the young plastid in spermatogenous, archesporial, and epidermal cells of *Polytrichum commune* and young androcytes of *Pellia epiphylla* showed staining similarities to the animal Golgi apparatus and believed that such might be associated with enzyme secretion, in the former case linked with starch production and in the latter with secretory granules. He did not think, however, that the two should as yet be considered homologous.

Sass ('34) offered further evidence of a Golgi apparatus in the Basidiomycetes, although he failed to associate it with any property of motility as in animals or with the plastidome reported by Weier. The "*nebenkern*" in *Coprinus sterquilinus* was found to divide into four "Golgi crests" which eventually became situated at the tip and presumably cooperated in the formation of sterigmata and in nuclear migrations. These elements resemble superficially the centrosomes of Maire ('02). His results were especially interesting in that he used various new fixatives absolutely free of osmic acid, which eliminated possible errors due to diverse osmophilic substances.

2. INVESTIGATIONAL RESULTS

Because in all cases investigated the mechanism of spore formation has appeared to be similar, the results shall be out-

lined in general for the whole group. Plate 1 includes exemplary types selected at random and the figures here mentioned refer to that plate.

The incipient phialide shows a blunt tip which is thicker than the lateral walls and stains darker, be it typically flask-shaped as in *Gliocladium fimbriatum* or essentially straight as in *Cephalosporium niveolanosum*. The dense granular contents do not extend quite to this tip since there is a hyaline area between (fig. 2). A chain of small round granules collect longtitudinally, and in fixed preparations show "tails," suggesting rapidly streaming protoplasm (fig. 3). These granules apparently merge into several small rods (fig. 4). Presumably at about this stage a drop of liquid is exuded terminally (figs. 5 and 6); at least in living cultures on hanging drops and agar slides these drops are infrequently noted on blunt hyphae. In the latter material the globule sometimes disappears as it is viewed and runs down the side of the phialide, producing the obviously different appearance of this structure, so often noted, as compared to the rest of the mycelium. In the hanging drops, with low magnifications the downward-hanging fructifications portray cephalosporia with spores literally swimming in the slime which here has not been disseminated by touching the glass. In the next step (fig. 7) a cross-section of the tip shows three granular rods, horizontally aligned, from each of which is a narrow short strip of cytoplasm leading to the protoplast proper. Figure 8 shows a split between four rods which "hold open the sac." The blunt tips seem to be especially resistant at the corners, and the adherence of rods to the sides probably increases this opposition so that the tip swells in the center slightly before splitting.

In the meantime, presumably associated with the exudation of liquid, the contents of the phialide have cleaved into a varying number of segments which at first are noted as regularly occurring cross-lines of granular dark-staining material, indicating regions of high surface tension. Later, narrow hyaline areas appear between the segments which round up at the edges. At the very base of the phialide are a varying number of small vacuoles. The spore, clad only in a fine membrane, be-

gins to protrude from the rigid collar of the phialide whose narrowness considerably retards the process (figs. 9 and 16). As if forced out by pressure from the increasing vacuoles at the base, the spore becomes somewhat constricted, as indicated by its tendency to round up on emergence, and causes the included end to become increasingly convex. Finally, after the greater part has emerged, the rest of the spore snaps off, and is immediately followed by a successor. This process is relatively slow, under optimum conditions only three spores being emitted from a phialide during seventy minutes (fig. 17). The exuded slime and the moist surface of the spore hold it near the phialide. In the spore ball it cannot escape until moisture disseminates the mucilage. Very thin sections of paraffin material seem to indicate that the spore is abstricted as is the yeast bud (fig. 15). This appearance may be explained by assuming that one of the granules at the tip really stays in the center of the phialide and draws the cytoplasm as a narrow stream into the spore; and in some thin sections it is impossible to see any lateral granules. Moreover, young phialides show blackish regions at the tips which probably represent central granules.

Since the greatest diameter of the phialides averages only 2 μ and the contents are granular, it is impossible to make out nuclei in the celloidin material, but in the thinner paraffin sections each cell and endoconidial segment appear to be uninucleate. The energid is but slightly larger than other dark-staining metachromatic granules throughout the cytoplasm. No definite mitotic stages were espied. The fine stream of protoplasm passing into the spore stains very darkly, perhaps due to a passing nucleus or to compression and rapid streaming. Sometimes mature spores show numerous refractile granules, at other times a more definite accumulation which is regarded as a true nucleus. After emergence, the spores form a thin wall around the membrane, swell doubly or triply, and germinate after several days. In some cases they appear to be germinating while still within the ball (pl. 5, fig. 16). Since the mucilage swells upon the addition of water, when favorable moisture conditions occur the spores are released both for dispersal and germination. In the meantime, they have been

protected by the slime. Thus cytologically the conidia in these species are haploid representatives of haploid individuals, apparently capable of indefinite existence without fertilization.

While the spores are being released the vacuolar area within the phialide multiplies conspicuously. The latter is increasingly contorted and devoid of protoplasm (especially noticeable in the larger single conidiophores of *Clonostachys Araucaria*). Doubtless, the vacuolar force, as well as the mechanical one of twisting, aids in the liberation of the spores. Neither by differential staining nor polarized light were any essential differences in the chemical nature of the phialide and ordinary mycelium observed.

Young tips of ordinary hyphae were more clearly observed in hanging drops than on agar. They are always more or less pointed and of the same consistency as the rest of the wall. Vacuoles extend quite close to the apex and contain one or several very active and large dancing bodies (pl. 3, fig. 21). Buller ('33) mentioned highly refractive particles (Woronin bodies) in *Ascobolus pulcherrimus,* on both sides of the septa and in terminal cells in a dispersed condition. In older cells they were said to settle down in the walls of the vacuoles, never in the protoplasm proper, and were associated always with the sap cavity, even passing through septa with it. These may be identical with the large dancing bodies, or even the larger septal granules (pl. 3, fig. 20), although the author does not think the latter two are the same. In fact, there seem to be dancing bodies which appear dark even *in vivo,* and cytoplasmic granules which show only in fixed preparations. The crowded vacuoles with small metachromatic granules visible between them soon occupy approximately the whole cell except for a narrow peripheral layer and a cytoplasmic region in the center where the nucleus is hung (pl. 3, fig. 19). Around all aerial hyphae on agar slides was noted a fine layer of mucilage, which indicates that apparently such exudation is normal in the life of the organism, and differs only in quantity in the phialide.

For comparison, a cursory examination was made of living material of *Scopulariopsis brevicaulis* (Sacc.) Bainier, a

species whose spores are held together in chains by disjunctors. The process agrees very well with that shown by Scaramella ('28) in *Penicillium digitatum* Sacc. The terminal uninucleate segment puts down an internal wall as a chlamydospore does, and by its swelling ruptures the external wall at the shortest diameter of the ellipsoidal spore which remains adherent at the base and connected with the rest of the phialide. This leaves empty a lens-shaped connective between the chain of spores abjointed similarly and successively. Meanwhile, there has been a slight cleavage of the protoplasm into a number of segments within the phialide, so that although only one spore becomes encysted at a time the potential ones are already present as in the phialide of the cephalospore. The main differences in the latter are the lack of resistant spore walls and method of rupture of the phialide, whose functions, as well as that of the disjunctor, are taken over by the mucilage. Comparative cytological data would be interesting.

3. INTERPRETATION

It seems probable that the incipient phialide eventually forms a rigid tip which is no longer capable of stretching and of intussuscepting material. The contents of the cell are continually swelling due to vacuolar as well as osmotic pressure and to surface tension, indicated by accumulation of granular material which eventually causes a rupture. In the meantime the granules have strengthened the corners so that the middle of the tip is stretched and broken and the size of the opening is considerably narrowed. The exudation of the mucilage, whether effected by the granules or not (though such seems likely, and it must come from the living protoplasm itself), doubtless makes possible the cleaving of the protoplasm as has been suggested in the basidium and sporangium. Whether the terminal granules represent centrosomes or Golgi bodies, the author is not prepared to state; but at any rate they must represent kinoplasmic elements similar to those of Scarth ('27). They may be an aggregation of chondriosomes whose significance is not clear.

Further, the mucilage production may be compared with guttation in the angiosperms (Wilson, '23), where there were found 220–1030 ppm. of organic material, mostly nitrates and nitrites, in the exudate. This indicates that occasionally colloidal material can be passed through intact walls. The terminal wall was not broken in the phialide at first, since it was sometimes visible within the droplet. The theories that the slime arises from between the membranes, as suggested by Knoll ('12) in cystidia, or by deliquescence of the middle lamellae (Brierley, '15) or of spore walls themselves (deBary, '84), are plainly inapplicable here.

Granules such as those occurring terminally in phialides are not noted in the ordinary mycelial tips, although similar (?) ones (pl. 3, fig. 20) are discernible in ordinary cross septa, perhaps exercising a function associated with the protoplasmic connectives.

The cleavage itself is largely a surface tension phenomenon and is doubtless often only partial, i. e., the cleaved parts occur only in small numbers towards the tip of the phialide and this process may be repeated; hence all the nuclear divisions do not have to take place at once. Such is exemplified in embryological studies of cleavage of animal eggs. Possibly, due to the water-holding capacity of the nucleus, liquid is exuded equidistant between the energids where increased surface tension is noticeable in fixed material by a layer of basophilic granules. Buller's physical theory would explain the size of the segments (cf. p. 10 of the present paper). The spores are released by the vacuolar pressure in the lower part of the phialide. Cohesion and surface tension factors, associated with a narrow rigid opening and the tendency of an elastic portion of a spore to round up when the pressure is released, assist the process. The size and shape of the spore are doubtless dependent upon the extent of the phialide and its opening as well as upon the amount of cytoplasm included in the endoconidium, all being explainable upon a purely physical basis. That the species of *Cephalosporium* have the longest narrowest spores is due to a comparatively straighter phialide which makes the endoco-

nidial column more extensive. Extrusion thus takes longer, and the membrane or new cell wall becomes more or less rigid before the spore is completely released.

The purely physical force of pressure must also be assisted by that of twisting caused perhaps by spiral streaming of the protoplasm. Although the author has seen no previous mention that this force assists in spore dispersal, Castle's ('34) reference to it in growth is suggestive. He states that the twisted or helical forms of growth are generally due to the resolution of two growth vectors: one longitudinal and one rotational. Oort ('31) had measured simultaneous growth and twisting along the axis in the coenocytic spore-bearing cells of *Phycomyces* and found at 17.5° C. an average rate of elongation of 39 μ a minute and an average rate of rotation of 3.7 degrees a minute. The angle at which micelles were incorporated in the wall was about 6 degrees. The steepness of the growth spiral was not structurally fixed but was reversibly altered (reversed or abolished) by change of temperature. The majority of the cells grew in the form of a right-handed helix, i. e., left-handed thread on a screw. The rapidity with which the angle of coiling changed seemed to refute the interpretation of altered proportions of different types of isomeric molecules. It may be that spiral streaming of protoplasm helps in the phialide twisting, but this is purely conjectural since no proof has been obtained.

The eventual collapse of the phialide results from the lack of compensating turgor pressure from the end wall which has become open (also an aid in the spiral twisting). Such a condition is less noticeable in the typical penicillate fructifications which have the most minute openings, especially in comparison with those of the rest of the phialide, than in the broader, straighter, and simple ones of *Clonostachys Araucaria*. If the spores were merely produced acrogenously there would be no exhausted remnant; moreover the endogenous condition here is more similar to that in the basidium and less like that in the ascus because there is no residual epiplasm.

The slime forms a protective covering around the delicate

thin-walled spores until optimum moisture conditions arise, when it not only makes the spores available for germination but also causes considerable dispersal due to the colloidal swelling accompanying its solution in water. Because of this miscible property the amount persistent around the spores will depend upon the humidity of the atmosphere. In very dry conditions the external layers tend to become almost rubbery, as ascertained by Cooke and Ellis ('78) in the solid slime balls on herbarium specimens of *Penicillium repens.*

The submerged cephalosporia of *Cephalosporium Serrae* appear to be homologous with the aerial ones except possibly for a greater tendency to irregularity in shape due to the confining action of the medium. In *Torulopsis mucilaginosa,* on the other hand, the whole growth is obviously a budding pseudomycelium and actual production of endoconidia was not observed. In *Cephalosporium symbioticum,* the cephalosporia attached to the short laterals more nearly simulate the fructification of *Allescheria Boydii* than that of *Cephalosporium.* Since this condition occurs in *Torulopsis mucilaginosa* only in submerged growth, it is an abnormality, and whether it may indicate a foreshadowing of the conditions in the regular forms or a reversion from an advanced to a reduced type (the yeast) is still a question. The latter view is the more logical.

In agreement with Wakayama ('31), the present data indicate that the conidiophore is an asexual organ of the haplont producing uninucleate spores which repeat the life cycle without variation except for secondary asexual spore forms. The occasional conjugation of conidia is without significance so far as is known.

B. OTHER CONIDIAL FORMS ENCOUNTERED

Omnipresent in this group are terminal or intercalary chlamydospores whose new cell walls are put down inside the parent cells. The production of chlamydospores is ordinarily associated with adverse conditions, and dissemination occurs when the adjacent cells die. Besides these, in *Gliocladium fimbriatum* and *G. deliquescens* large terminal cells rich in oil

droplets are formed, which differ from chlamydospores in their relatively thinner walls and capability of immediate germination as evidenced in hanging drop cultures. For such, the designation akinete has been adopted from algological terminology. Moreover, there are noted within the medium enlarged round to ovoid terminal cells beyond which the hypha may grow again (pl. 3, fig. 30). Their subsequent development was not followed, but when observed they were thin-walled. They may possibly be aborted ascogonia, and Thom has referred to them as due to the same impulse as that causing submerged cephalosporia. If they function as chlamydospores, at least their method of formation is somewhat irregular.

The peculiar short-stalked spores produced promiscuously over the terminal clubs in *Clonostachys spectabilis* cannot be discussed in detail since the culture died early, but they are obviously not comparable to the other forms studied. A superficial resemblance between spores and the warty structures of the phialides of *Clonostachys Araucaria* may have caused Oudemans ('86) to include that species in *Clonostachys*. However, the warts are not spores but thickenings on the wall, not uncommonly seen in fungi and are incapable of germination.

Infrequently oidia were noted in *Gliocladium Vermoeseni* (pl. 4, fig. 11). The significance of these secondary spore forms is obscure. If such occur in *Penicillium* and *Aspergillus,* Thom ('26, '30) did not mention them, unless the "hülle" cells first cited by Eidam ('83) in some species of *Aspergillus* could be considered such. It is obvious that hypnosporic types of spores are produced in response to unfavorable conditions, especially where the colony has exhausted the medium and an indefinitely resting spore is needed.

IV. PHYSIOLOGY

A. COMPARATIVE CULTURAL CHARACTERISTICS

In table 1 are listed most of the organisms studied on a representative number of media, with brief notes regarding color, surface, and microscopic characteristics. Some correlations

Organism	Nitrate pH 5.5	Czapek pH 4.43
Allescheria Boydii	Gull-gray, powdery, submerged; single spores.	Gray-white, flat aerial.
Cephalosporium kiliense	Apricot-buff, cerebriform; chlamydospores; submerged cephalosporia.	Orange, rough, heaped, edges submerged.
C. nivcolanosum	Shrimp-pink, flat, wrinkled; chlamydospores; submerged cephalosporia; long coremia.	Bluish-white, flat, with radiate striations.
C. rubrobrunneum	Orange-pink, flat, corrugated; submerged cephalosporia.	White, edges submerged, later cottony.
C. Serrae	Gull-gray, short-fibrous; submerged chlamydospores and cephalosporia.	White, heaped, rough, edges submerged.
C. Stuehmeri	White, short, rough surface, submerged; many cephalosporia.	White, flat, granular, fimbriate.
C. symbioticum		Pale pink, mostly submerged; few coremia; many cephalosporia.
Clonostachys Araucaria	Citron, in medium too, powdery, submerged; few aerial cephalosporia.	White, slightly raised, granular center, orange below; cephalosporia.
Clonostachys Araucaria var. *rosea*	Primuline-yellow, in medium too, powdery, submerged; few aerial cephalosporia.	White-opaque, discrete tufted colonies; few cephalosporia.
C. Araucaria var. *confusa*	Orange-pink, powdery; cephalosporia.	Pink, raised, fuzzy.
Gliocladium deliquescens	White, green dots, mostly submerged scattered threads.	White, fuzzy; submerged cephalosporia.
G. fimbriatum	Dark green, medium yellow-brown, felted; cephalosporia and akinetes.	Dark green, white edges; cephalosporia.
G. Vermoeseni	Pink powdery; many cephalosporia.	White, sparse mycelia; pink cephalosporia.

TABLE I

COMPARATIVE CULTURAL CHARACTERISTICS IN VARIOUS MEDIA

ek .43	Potato dextrose pH 5.7	Corn meal pH 6.0	Gorodkova-Maneval pH 6.8	Sabouraud's dextrose pH 5.6	Lactose br pH 6.8
at aerial.	Gray-green, black below, wrinkled; coremia; single spores.	Gray, powdery; single spores.	Tan-gray, flat, matted, filamentous; single spores.	Pale-gray, long, fuzzy; many single spores.	Gray pellicle of diating colonie
h, heaped, erged.	Deep orange, furrowed; cephalosporia; chlamydospores.	Pale pink; submerged chlamydospores and cephalosporia.	Pale pink, flat, moist, sterile.	Pink, corrugated; coremia; small cephalosporia.	Pink ring, liquid
flat, with tions.	Yellow-white, heaped in center, hairy projections, sterile.	White, wrinkled; many cephalosporia.	Pale tan, heaped, hairy protrusions; chlamydospores.	Few cephalosporia.	White yeasty rin turbidity.
submerged, y.	Coral-pink, corrugated; few cephalosporia.	Deep pink, flat, moist; cephalosporia.	Pale pink, wrinkled, much submerged, sterile.	Pale orange, flat, moist; few cephalosporia.	White ring, sligh ity.
ed, rough, erged.	Steel-gray; many chlamydospores; few cephalosporia.	Silvery-gray, powdery; much submerged chlamydospores; few cephalosporia.	Gray-powdery, flat, wrinkled; chlamydospores.		Downy white pell
anular, fim-	Pale pink; nodular coremia; many cephalosporia.	Pale pink, slightly elevated, dry; many cephalosporia.	Opaque, round elevated, radial furrows, sterile.	White, moist; some coremia; few cephalosporia.	White yeasty ri bidity.
nostly sub- w coremia; losporia.	White, matted with pink coremia, medium grass-green.	Chlamydospores; submerged cephalosporia.	Pink, flat, wrinkled; submerged cephalosporia and chlamydospores.		Yellow-green pink submerged
tly raised, nter, orange alosporia.	Pink, cottony; many chlamydospores; few cephalosporia.	Pale pink, scattered, small dry heaps; cephalosporia; chlamydospores.	Cephalosporia.	Pale pink, dry, powdery; many cephalosporia.	Powdery white some hyalin growth.
e, discrete onies; few ia.	Chalky white, heavy, flat-matted growth, sterile.	Opaque, flat; cephalosporia.	Chalky-white, matted, coarse; chlamydospores; cephalosporia.		White yeasty rin turbidity.
fuzzy.	Pale pink, flat with powdery tufts; cephalosporia.	Pink, scattered tufts, dry; cephalosporia.	White, rough, matted; cephalosporia.	Pale pink and white, downy.	Pink-white, pow licle, liquid cle
; submerged ia.	Blackish-green, medium brown; aerial cephalosporia.	Green dots scattered, sparse growth; loose spores.	Sterile.	White; many cephalosporia.	Gray-green surf licle, liquid clear, lar no cephalospor
white edges; ia.	Deep green, medium tan, matted, moist; many cephalosporia.	Green, moist; many cephalosporia.	Yellow-green, powdery, moist; cephalosporia.	White, medium yellow-green, cottony.	Pale green, feat licle, hyphae too.
se mycelia; losporia.	White, fibrous; chlamydospores; submerged cephalosporia.	Pink, powdery, elevated; few small cephalosporia.	White, powdery, sterile.	Transparent, scattered.	Pink ring, liquid

TABLE I

OMPARATIVE CULTURAL CHARACTERISTICS IN VARIOUS MEDIA

Corn meal pH 6.0	Gorodkova-Maneval pH 6.8	Sabouraud's dextrose pH 5.6	
powdery; single a.	Tan-gray, flat, matted, filamentous; single spores.	Pale-gray, long, fuzzy; many single spores.	Gray diat
ink; submerged ydospores and losporia.	Pale pink, flat, moist, sterile.	Pink, corrugated; coremia; small cephalosporia.	Pink
wrinkled; many losporia.	Pale tan, heaped, hairy protrusions; chlamydospores.	Few cephalosporia.	White turb
ink, flat, moist; losporia.	Pale pink, wrinkled, much submerged, sterile.	Pale orange, flat, moist; few cephalosporia.	White ity.
gray, powdery; submerged chlam- ores; few ceph- oria.	Gray-powdery, flat, wrinkled; chlamydospores.		Downy
ink, slightly ele- , dry; many ceph- oria.	Opaque, round elevated, radial furrows, sterile.	White, moist; some coremia; few cephalosporia.	White bidi
ydospores; sub- cephalosporia.	Pink, flat, wrinkled; submerged cephalosporia and chlamydospores.		Yello pink
k, scattered, small eaps; cephalospo- hlamydospores.	Cephalosporia.	Pale pink, dry, powdery; many cephalosporia.	Powd son gro
, flat; cephalospo-	Chalky-white, matted, coarse; chlamydospores; cephalosporia.		White turl
scattered tufts, cephalosporia.	White, rough, matted; cephalosporia.	Pale pink and white, downy.	Pink- licl
dots scattered, e growth; loose a.	Sterile.	White; many cephalosporia.	Gray- licl qui no
moist; many ceph- oria.	Yellow-green, powdery, moist; cephalosporia.	White, medium yellow-green, cottony.	Pale licl too
owdery, elevated; small cephalospo-	White, powdery, sterile.	Transparent, scattered.	Pink

trose	Lactose broth pH 6.8	Sabouraud's Conservation pH 7.0	Glycerine pH 6.9	Carrot plug
fuzzy; rea.	Gray pellicle of small radiating colonies.	Gray-violet, black, flat, powdery.	Dark gray, corrugated; single spores.	Gray-green, short, fuzzy.
; cor- alospo-	Pink ring, liquid turbid.	Pink, moist, much wrinkled.	Strawberry-pink, slightly wrinkled; many coremia.	Pink, tallowy, with radiating coremia.
	White yeasty ring, slight turbidity.	White, round elevated centers and spreading hairs.	Seashell-pink, rope-like, with coremia.	White, tallow-like; few coremia.
moist; a.	White ring, slight turbidity.	White-silvery, hard cerebriform colony.	Frosted seashell-pink, cerebriform; many cephalosporia.	White, tallowy.
	Downy white pellicle.	Yellow, powdery tops, wrinkled.	Pale pink, cottony, slightly wrinkled.	White, tallowy, some fuzz; some coremia.
ie cor- alospo-	White yeasty ring, turbidity.	White, hard, wrinkled shell, orange below; coremia.	Pink, wrinkled below; coremia.	White, tallowy.
	Yellow-green pellicle; pink submerged hyphae.	Pink then black, mostly submerged; cephalosporia and chlamydospores.	Orange coremia and pink-yeasty then black; chlamydospores.	White powdery.
wdery; ria.	Powdery white pellicle, some hyaline basal growth.	Pale pink, cottony; cephalosporia.	Shrimp-pink, downy, umbonate, wrinkled below.	Pinkish-white, short-haired fuzz.
	White yeasty ring, slight turbidity.	White, yellow below, wrinkled.	Pink center, rough, hairy, wrinkled below; cephalosporia.	White, small cottony, sparse growth.
white,	Pink-white, powdery pellicle, liquid clear.	White, matted, yellow below, wrinkled.	Pink-orange, thick-matted, wrinkled below; few cephalosporia, swollen thin-walled cells.	Pink, fuzzy, good growth.
alospo-	Gray-green surface pellicle, hyaline below, liquid clear, large cells; no cephalosporia.		White cottony center; cephalosporia at edge.	Delicate green fuzzy growth.
ellow-	Pale green, feathery pellicle, hyphae in liquid too.		Meadow-green, edges white, rapid flocculent growth, medium olive-yellow; many cephalosporia.	White hairy growth.
ered.	Pink ring, liquid clear.	Pink spore balls, white cottony hyphae.	White mycelia; lavender spores; rapid growth.	No growth.

may be drawn here, indicating as favorable media the glycerine, Sabouraud's dextrose, and Czapek's agars; and as unfavorable, nitrate and Gorodkova-Maneval agars; as less so, corn meal and potato-dextrose agars, carrot slants, and liquid media. Growth on the first group is typified by abundant large, aerial mycelia with comparatively few types or numbers of spore forms, deeper internal coloring with little if any exuded pigment, much guttational water, and a convoluted colony. Unfavorable conditions are recognized in sparse surface growth of long slender filaments, a tendency to submerged mycelium, relative increase in reproductive bodies such as chlamydospores, conidia, and coremia, dry powdery growth, paler or tending toward yellow rather than orange pigmentation endogenously, with frequent exogenous coloration of the medium.

In the next section some of the prominent factors and theories for cultural deviations which seem especially applicable in the case at hand will be more fully discussed.

B. FACTORS INFLUENCING MORPHOLOGY AND PHYSIOLOGY

Of the numerous variables possibly relevant, the present discussion is limited to hydrogen-ion concentration, surface and oxygen tensions, and kinds of nutrients. Temperature, doubtless influential, will not be considered, since all were grown at 30° C.

1. Hydrogen-ion concentration.—It must be remembered that the hydrogen-ion concentration refers to the relative acidity or percentage of dissociation as contrasted with the total acidity; although the latter factor, as well as the chemical nature of the acid itself, also influences growth. Moreover, microorganisms contain natural buffers and have to a limited extent the power to adjust the pH of a medium toward the optimum as a result of their activity.

The media utilized covered a wide natural range of H-ion concentration, from Raulin's (pH 4.15) to Endo's (pH 7.5), with growth upon all. An artificial series from pH 3.8 to 7.6, maintained upon glycerine agar (table II), gave the most striking results in the tendency towards submerged growth at the

higher concentrations of pH. At pH 4.4 and below and at pH 7.6 the media did not solidify. It is interesting that *Gliocladium deliquescens* grew in the liquid at the higher acidities, but formed a pellicle at the higher alkalinities. On the other hand, *Torulopsis mucilaginosa* grew extensively and normally upon wort agar (pH 4.8) but with mainly submerged cephalosporia upon nitrate agar (pH 5.5); whereas the true molds showed an inundated state upon the latter and abnormal aerial conidiophores even upon wort.

Organisms listed as *Monilia albicans* have been extensively used in physiological studies. Marantonio ('93) stated that at the higher acidities there was a greater quantity of mycelium; and that there was a tendency to filamentous growth on liquid, with sprouting almost exclusively upon solid media, or hyphae only on old cultures. According to Fineman ('21), mycelial growth was better under low surface and oxygen tensions, but the yeast form predominated upon solid media, simple carbohydrates, and a low acidity. Mallinkrodt-Haupt ('32) stated that the yeast form predominated in a strongly acid medium, whereas the filamentous occurred in a very alkaline one; and he also mentioned that H-ion concentration influenced the metabolism, enzyme actions, morphology, toxin production, and immunological characteristics.

Buchanan and Fulmer ('28) found that some of the molds could tolerate a range of pH from 1.6 to 11.2, but that there was an optimal section with atypical growth deviations on either side. With fermenting yeasts alcohol was formed under acid conditions, and glycerol as an intermediary under alkaline ones.

In *Endomyces capsulatus,* Moore ('33) said that in acid media the tendency was towards abundant budding, numerous conidia, and longer narrower cells; contrarily, on alkaline substrata there was an inclination towards shorter thicker cells with yeast-like formations and fewer conidia.

Hewitt ('33) said that the pH had an effect upon the oxidation reduction system, which resulted in an alteration of the ionic equilibria. Clark ('22) thought that the H-ion concentration functioned chiefly as a conditioning agent and was only in-

TABLE II

EFFECT OF HYDROGEN-ION CONCENTRATION—GLYCERINE AGAR

Organism	pH 3.8	pH 4.4	pH 5.0	pH 6.0	pH 6.4	pH 7.0	pH 7.6
Allescheria Boydii	No growth.	No growth.	Gray, velvety, somewhat submerged, wrinkled.	Very slight growth.	Gray-velvety growth.	Luxuriant gray down, black center, moist.	No growth.
Clonostachys Araucaria	No growth.	No growth.	Fine mycelium, pink, powdery; one coremium.	Pink, fluffy, powdery.	Pink and white down, wrinkled, moist.	White down becoming pink, powdery, dry.	No growth.
C. Araucaria var. *confusa*	Slight growth.	No growth.	Pink-orange, powdery; few coremia.	Pink, cottony, quite coarse.	Very cottony and pink, moist.	White then salmon, downy.	Small amount of submerged growth.
Cephalosporium Serrae	No growth.	No growth.	Pink, coarse growth, soon becoming black with chlamydospores.	Heaped compact colonies.	White, wrinkled, cottony; coremia.	White, tufted.	No growth.
Gliocladium deliquescens	Submerged growth; cells thin-walled, granular, short, contorted, with intercalary ones of swollen walls; no cephalosporia.	Colorless growth in liquid; cells less contorted and longer, granular; some cephalosporia.	Mostly submerged growth, white with green dots.	Fine white surface growth with green slime balls, akinetes; cells long and narrow, heterogenous contents.	Flocculent white growth with green slime.	Flat dull surface growth and somewhat submerged.	Heavy pellicle and radiating hyaline hairs; cell walls thick, hyaline, contents granular; few terminal chlamydospores; no cephalosporia.

directly concerned with chemical transformations closely related to life processes.

2. Surface tension.—Surface tension is the cohesional pull of the internal and adjacent molecules upon those of the surface layer. It is particularly important in the life of the organism in relation to adsorption and absorption. This tension produces surface energy, which, unhindered, effects the assumption of minimal surface for a given volume, i. e., a sphere. At the interfaces which are sites of chemical and physical activity, surface tension is constantly being opposed. Anything causing a lowering of surface tension will permit a larger surface; for example, mycelial growth will be favored, but under the other condition the cell size will be limited and yeast-like proliferation result. The appearance of filamentous forms in liquids is probably a reaction due partly to surface tension, and represents unfavorable adaptation to an unfamiliar substratum.

Artificial reduction of surface tension has been secured by Fineman ('21) with the addition of sodium ricinoleate to a medium with dextrin solutions, but not those containing galactose. Glycerine has also been found to lower surface tension and increase mycelial growth. Upon the basis of similar observations, Czapek ('11) was led to stipulate the necessity of lipoids in the surface membrane. Surface tension is greatly affected by the polar orientation of interfacial substances, the amount of ionization, and the pH (which determines the basic or acidic activity of amphoteric proteins).

Larson ('21) attributed his success in growing normal pellicle-forming bacteria on the bottom and vice versa by lowering or raising surface tension, to the non-wetting properties of the lipoids in the surface pellicle. Buchanan and Fulmer ('28) reported similar results in making *Clostridium tetani* grow superficially, but they suggested that the surface-active solutes concentrated in the surface boundary might also cut down the solubility of oxygen. They also mentioned the tendency of *Bacillus subtilis* to sporulate in media of low surface tension. Oxygen tension, availability of nutrients, etc., may overbalance or at least modify the effects of surface tension.

3. Oxygen relationships.—The aerobic and anaerobic states are dependent upon the oxygen relations. Topley and Wilson ('29) stated that anaerobes were not supersensitive to oxygen itself, i. e., that oxygen was not toxic, but that hydrogen peroxide was produced abundantly in the presence of free oxygen for whose destruction no catalase or equivalent system was present. This theory also explains why anaerobes can sometimes be cultivated with an aerobic form in air, the latter destroying the peroxide.

Fineman ('21) found mycelium formed in *Monilia albicans* on surface agar slants cultured in carbon dioxide. The usual correlation of budding and high oxygen tension and of mycelium and low oxygen tension was noted, but there were exceptions. According to Fuchs ('26), reduced oxygen tension favored sporulation in yeasts. Dodge ('35) stated that lowered oxygen tension favored hyphal production to some extent.

The metabolic relation to oxidation is also intimately associated with the substratum. Stephenson (Buchanan and Fulmer, '28) found the normally aerobic *Bacterium coli (Escherichia coli)* developing anaerobically during the first stages of glucose breakdown, and thereafter aerobically. However, if grown in pure oxygen it utilized such from the beginning, nor was there any increase in the ratios of glucose decomposition, but the active metabolism persisted longer than in the former case and the acidity increased more slowly. Thus the apparent effect of the increased oxygen was to induce further breakdown of some acid product of the fermentation of glucose. An increased oxygen tension accelerated the respirational rate when dextrose was replaced by ammonium-lactate, and made possible the derivation of carbon from salts of acetic or succinic acids but under aerobic conditions only. In a synthetic medium of simple organic acids (such as lactic, succinic, fumaric, or pyruvic) to which nitrate was added, the organism could grow anaerobically by utilizing the reduced nitrate as a hydrogen acceptor or oxygen donator. Buchanan and Fulmer ('28) further noted that in *Bacterium coli (Escherichia coli)* and *Clostridium Welchii* smaller amounts of carbon dioxide were produced aerobically than anaerobically.

Hewitt ('33) offered an explanation of aerobisis and anaerobisis on the basis of oxygen-reduction potentials in place of the view that anaerobes have no catalase. He showed that aerobes (such as staphylococci, streptococci and pneumococci) reduced the broth medium whereon they were cultivated, which had an initial potential of almost –0.3 volts, to as low as –0.15 to –0.20 volts. Anaerobes, on the other hand, could not begin growth on such a medium, but with pieces of meat in the broth the potential became reduced to –0.2 volts, due to a reversible oxidation-reduction system therein. Such potential was not changed by staphylococci which had reached their minimum, but was lowered by anaerobes to a limit of –0.4 volts. The preliminary reduction of the medium might be effected artificially by four methods: (1) hydrogen in an anaerobic jar, (2) pure nitrogen, (3) various chemical-reducing agents, (4) growth of aerobic organisms.

4. Kinds of nutrients.—Besides the above-mentioned interrelations of oxidation and nutrient necessities, there have been some definite observations regarding the nature of the latter alone.

Talice ('30), in his extensive study of media and conditions favoring the formation of hyphae, found that mycelium was formed the first few days and then mainly yeasts, with the filamentous form only in contact with the agar surface. Surface tension may enter here and oxygen phenomena, since the inside of a colony is under reduced oxygen pressure. He noted that normal yeast forms produced hyphae with dextrin peptone media or glucose, and to a lesser extent with protein. Incidentally, he regarded the yeast state as a senescent one.

Plaut ('13) found yeasts in sugar-rich media and mycelia in sugar-free nitrogenous substrata. Buchanan and Fulmer ('28) spoke of the "protein-sparing" effect of dextrose. Topley and Wilson ('29) thought that there was no such effect, but rather an inhibition of bacterial growth, due to the rapid increase in hydrogen-ion concentration of those media containing a fermentable carbohydrate.

Further conclusions have been drawn from work with *Monilia albicans*. Roux and Linossier ('90) stated that the com-

plexity of morphological structure increased with the molecular weight of the food elements, i. e., yeasts in simple sugars, and filaments in complex carbohydrates. Such statements have not been fully confirmed. According to Mallinkrodt-Haupt ('32), the yeast form appeared in the tissues of the host, but in culture the mycelial one. Discordantly, Fineman ('21) reported both conidia (yeasts?) and mycelium in the host, but only the one or the other in culture. No explanation was offered but if such be true, the heterogenous nature of the internal environment may be responsible.

5. Discussion.—It soon becomes obvious, when trying to check physiological causes and effects, that there are many variables concerned, making it practically impossible to attribute a definite reaction to one alone. Nevertheless, some likely influences can be designated.

There is an optimum range of hydrogen-ion concentration between pH 5 and pH 7, and the best is around pH 6.4 for the majority of these forms. Below and above the range the growth is restricted and the relative number of reproductive bodies, especially chlamydospores, augmented. Moreover, the maladjustment is exemplified by atypical colony characters—i. e., submerged contorted growth at the higher acidities and aerial if scanty growth with increased alkalinities.

The forms studied show strong aerobiosis, tending to form pellicles on liquids, but are occasionally submerged with modified hyphal growth due to other more influential causes. If other conditions are unfavorable, however, all of the organisms may grow immersed even on a solid substratum. These molds seem to require media rich in both carbohydrates and proteins. The organic nitrogen foods are more easily assimilated than the mineral salts; and sugars better than starches. Glycerine is readily utilized.

Some of the present results may be explained as follows: the relative acidity may occasion the submerged growth in Czapek's medium; low carbohydrate supply in nitrate and Gorodkova-Maneval agar necessitates the utilization in the first case of nitrogen of a mineral nature and in the other of an organic nature, with comparable results; utilization of com-

plex plant carbohydrates *in situ* in corn meal, potato, and carrot, with increased production especially of coremia; strong aerobic tendencies evidenced by heavy pellicle, with the species of *Cephalosporium* best able to grow anaerobically if atypically submerged.

Although some have regarded the yeast type as a senescent primitive form (Talice, '30), present evidence would seem rather to indicate a reduced type. The yeast has become a highly specialized saprophyte, i. e., adapted to growth in sugar solutions. Such a habitat has effected extreme morphological changes which caused sexual degeneration, and the almost exclusive assumption of the budding state due probably to chemical and physical causes associated especially with surface tension and the nature of the substratum. Perhaps the status of oxidation-reduction potential in the sugar medium may elucidate upon the morphological effect. Hewitt ('33) lists carbohydrates as having an unknown reversible oxidation-reduction system.

The insertion of the yeast stage normally in a filamentous form is interesting too. For example, the smut sporidia propagate rather indefinitely by budding. Conidia of the Plectascales have also been found upon occasions to bud for various time intervals. Fuchs ('26) gave a review of the early concepts which regarded the sprouting interval as a part of the life cycle of filamentous forms. He found that *Aspergillus Oryzae,* especially on wort and wort gelatine, remained permanently in the yeast stage. Such data have not been duplicated to the knowledge of the author. The latter media have been found unfavorable to the molds, probably because of the large amount of sugar, reduced proteins, and also relatively high acidity. In pseudomycelial forms—*Monilia* in the general sense of the name—the yeast state is prevalent under favorable conditions, and the mycelial one under unfavorable situations according to Henrici ('30).

C. COLOR

The two dominant colors found in this group are green and red in various shades, although one organism was gray and

three were sporadically yellow. The first pigment seems quite constant under all conditions and is apparently in the mycelial protoplasm as well as the spores. In *Gliocladium deliquescens* optimal growth shows almost complete covering with a green slime whose color is doubtless due to the numerous spores included. *Gliocladium penicilloides* generally is chalky-white, but sometimes becomes pale yellow and on glycerine agar exudes the same pigment into the medium. A similar condition is present in *Clonostachys Araucaria* and its variety *rosea* on nitrate medium. The pigment is water soluble.

It is the various red and orange shades which attract particular attention. The pink pigment of *Gliocladium Vermoesenii* is obviously contained within the spores since the mycelium is a white flocculent growth. In the species of *Cephalosporium* the pigment is dispersed within the mycelium, but its intensity or mere presence is conditioned by varying factors of light, temperature, substratum, oxygen relations, associated probably with general metabolism. A few observations are to be mentioned here, although little experimental work was done upon their nature.

Cephalosporium kiliense when growing most luxuriantly in the light is a rich apricot-orange; but as the culture stales, or on media low in accessible nutrients, or in the dark, it fades to a pale yellow-orange or even almost white. *C. niveolanosum* is a paler pink at best and is white if kept in the dark (at the same temperature) for even a few days. The color of the *Torulopsis mucilaginosa* is more persistent, but in one case when a culture was placed in the refrigerator (13° C.) for two months it became white and remained so on all subsequent transfers.

An attempt was made to extract the pigment of *Cephalosporium kiliense* with various solvents. With concentrated sulphuric acid the specimen became blue-green immediately, and the liquid later had a cherry-wine color due perhaps to the dissolution of agar. Concentrated hydrochloric acid turned the solution a blue-green color which faded to yellow. Fuming nitric acid, as well as 20 per cent sodium hydroxide, dissolved the fungus and medium with no color reactions. There was slight solubility in chloroform as well as in hot 95 per cent

alcohol and in lactic acid. Entirely ineffective were petroleum ether, benzol, oxalic acid, salicylic acid, acetone, formaldehyde, and ethyl-ether. The pigment was especially soluble in acetic acid and upon evaporation yielded a yellow-orange mass which appeared amorphous under the microscope. This observation is insufficient to identify the pigment, although it seems related to xanthophyll. The reaction with hydrosulphuric acid was not the typical one of lipocyanin.

Color variations have been occasionally noted in fungi, and Zopf ('90) worked out some rather complicated methods of extraction and determination. Palmer ('22) stated that in some cases the reds and yellows were doubtless due to carotinoids, whereas in other cases they were not, as in chrysophanic acid. Pigments have also been cursorily noted in the bacteria, and it has been possible to separate rather constant strains, and at other times conspicuous color variants have been noted in a colorless colony or vice versa. Little or nothing is known of the chemistry or physiological purpose of pigments. Some theories have been promulgated: reserve of oily nature in the rust spores (Zopf, '90), a protective function to cellular enzymes (Went, '04), and an oxidation pigment. It may be that the pigmentation is largely associated with conditions of acidity and alkalinity in the medium, as Thom ('26) has suggested, yellow occurring in acid conditions and red in alkaline.

In the cases at hand—*Cephalosporium niveolanosum* and *C. kiliense*—it is possible that the pigment is a reserve and indicates excessive anabolism, but chemical investigation would be necessary to substantiate the view. A protective function seems quite logical whether for enzymes or the cytoplasm itself. The association with respiration seems unnecessary, since existence is possible without pigment in these species and in closely allied ones (e. g., *C. rubrobrunneum* and *C. Stuehmeri*, separated almost entirely upon the presence or absence of pigment). At any rate the problem is still an open one and possibly full of significance.

According to Henrici ('30), the pigment production in *Actinomyces* was more constant and also more striking in media of rather low nutrient value, which is the reverse of the present

findings except for *Clonostachys Araucaria,* where abnormal pigments are released in the medium. Colley ('31) enumerated changes in color of *Serratia marcescens* and *Azotobacter chroococcum* upon the addition of certain salts to Bacto-agar. In the latter case the color was supposedly due to the oxidation of tyrosin to melanin, the tyrosin being formed in the breaking down of proteins when the carbohydrate supply was insufficient and unsuitable; but on the addition of zinc salts the color production was due probably not to the exhaustion of carbohydrate but to unfavorable conditions preventing its use.

The intense yellow water-soluble pigments diffusing into the medium is obviously a metabolic product, perhaps the chrysophanic acid of Zopf. In the case of the nitrate medium, at least, it might indicate unfavorable growth, although it doesn't especially appear so for all media, certainly not with glycerine agar.

D. POLARIZED LIGHT

Since, as has been stated, the walls of reproductive cells are sometimes of different constitution from vegetative cells and since they might possibly have peculiar physical characteristics, they were studied with a polarizing microscope. Agar-slide cultures and hanging drops were employed under high power and oil immersion. No structural differences were detectible by this method, although the twisted nature of the wall in the phialide was most conspicuously brought out. Sometimes, in ordinary mycelia, adjacent cells were noted to refract differently. This is presumably due to the different orientation of the cells caused by twisting, but it may have been that some of them were beginning to form chlamydospores and hence were of different structure.

V. Fungi Imperfecti and Sexualty

Lindau's ('07–'10) treatment of the Hyphomycetes has been followed. The subfamily Cephalosporieae is separated from the Aspergilleae in that the spores are in balls in the former and in chains in the latter. The Aspergilleae include *Penicillium* and *Gliocladium. Clonostachys* belongs in the Verticillieae. *Allescheria Boydii,* also described in different develop-

mental stages as *Cephalosporium Boydii* and *Dendrostilbella Boydii,* because of its perithecial fructification, is placed in the Eurotiaceae of the Perisporiales. Clements and Shear ('31) have made *Allescheria* a synonym of *Eurotium.* The fertile state of *Gliocladium penicilloides* placed it in *Eurotium insigne* according to Winter ('87), but this disposition was later disputed by Matruchot ('95). Thom and some later workers have segregated the perfect forms of *Aspergillus,* as well as imperfect forms with similar conidia, in the Aspergillaceae, close to the Gymnoascaceae of the Plectascales.

A. EXPERIMENTS TO INDUCE SEXUAL FRUCTIFICATIONS

The author at first believed that the imperfect stages were merely labile and that perfect fructifications might possibly be produced by various special methods. In no case were these successful. After elaborate experimentation with a number of algae and other forms, Czurda ('33) came to the conclusion that copulation was caused only by pH and internal disposition, and since the latter was intangible, pH was the only factor subject to investigation. The present experiments did not indicate that there was a specific pH, at least one which was solely responsible. The usual media were employed to evoke fertile stages. Drying or maintaining at low temperatures merely showed the extreme longevity and resistance of these organisms. It was thought that by crossing nearly related species and varieties fertile stages might be attained. Therefore cross-inoculations were made of *Clonostachys Araucaria* and its varieties *rosea* and *confusa,* as well as of all of the species of *Cephalosporium.* The only noticeable result was an aversion of *Clonostachys Araucaria* and the variety *confusa.* The rest intermingled promiscously. Agar micro cultures, as well as Petri plate specimens, were observed, and although no particular hyphal fusions were noted, neither were there ordinarily signs of repulsion.

B. INTERPRETATIONS REGARDING LOSS OF SEXUALITY

Ramsbottom ('33) believed that heterothallism and saltation were responsible for the origin of the Hyphomycetes.

> Thus theoretically we may regard one of the Fungi Imperfecti as able to carry on the influence of the environment. If this is so it is possible that a permanent change may be brought about. . . . If such changes occur naturally then we may get differences which might throw heterothallism out of gear.

It was his suggestion that these should not be regarded as species but rather as form-species. Hansen and Smith ('32) held similar views on *Botrytis cinerea* which theoretically has a perfect stage in *Sclerotinia Fuckeliana*. They discussed previous explanations as (a) impure material, i. e., a complex of established types on the host which must be separated, (b) mutation, (c) hybridization, (d) microconidia—some possible significance but little evidence that they even have enough power of germination to play an important rôle, (e) anastomoses, hyphal fusions, heterocaryosis, i. e., condition of nucleus containing two or more genetically different nuclei, (f) mixochimaera (no cytological evidence of this yet). Their cytological studies showed that the mycelial cells and conidia were multinucleate. Further analyses of types were made by 128 single spore cultures, from which were selected 8 showing the greatest morphological variation. Twenty-five single spore cultures were made of these and a record kept for a number of generations. Hansen and Smith concluded that the regularity and completeness with which the homotypes separated from the heterotypes indicated that the character-determining elements were discrete units of limited number, suggestive that the basic unit of the individual was the nucleus and not the cell. Therefore a multinucleate spore, a colony really, cannot give rise to a genetically pure culture unless all of its nuclei are genetically identical. Gregory ('35) suggested the following explanation for loss of sexuality in the dermatophytes, namely, environmental factors, elimination of sexuality (negative adaptation to parasitism), and heterothallism.

Although the species here studied are uninucleate, heterothallism may well play a part. It is not unlikely that there are many races, new ones constantly being formed by the mechanism of hyphal anastomoses, and some of these are actually antagonistic although morphologically very similar. Perhaps some compatible strains have died out, leaving imperfect

halves to vegetate ceaselessly unless by saltations new harmonious ones shall arise.

VI. MEDICAL ASPECTS

A. PATHOGENICITY

Of the forms isolated from skin lesions—*Allescheria Boydii* and the species of *Cephalosporium*—there is some doubt as to real pathogenicity. Of the *Cephalosporium* species treated by Dodge ('35), only *C. Serrae,* of keratomycosis of the eye, and *C. Doukourei,* of a gummatous lesion, are said to be pathogenic to laboratory animals.

Allescheria Boydii, isolated by Boyd and reported by Shear from a case of granular mycetoma of man, was thought by them to be a contaminant and not an anaerobic form because of its peculiar morphology within the foot and its inability to infect laboratory animals. A number of other Hyphomycetes, including species of *Indiella, Glenospora, Scedosporium, Aspergillus,* and *Penicillium,* have been reported as etiological agents of maduromycosis. At present it would seem that such were purely accidental contaminations, which may be fatal but are not naturally so.

The author secured species of *Penicillium* and *Aspergillus* frequently from eczematoid infections at the Barnard Free Skin and Cancer Hospital, and the *Cephalosporium symbioticum,* with the intimately associated *Torulopsis mucilaginosa,* from a particularly severe case of dermatomycosis. She also isolated the latter with a similar co-form which was eliminated in subsequent dilution cultures from a case of pemphigus vulgaris. Apparently the same red yeast was found by Engelhardt ('27) in a case of pemphigus. He illustrated the organism as it grew in the host, i. e., filamentously, similar to the condition here found on nitrate agar, although no cephalosporia were shown. Whether such organisms are purely saprophytic secondary or primary invaders, or necessary symbionts of forms better believed to be pathogenic (Hartmann, '26), in the present case of a *Cephalosporium* and *Torulopsis mucilaginosa,* is as yet unknown; but immediate evidence at

least indicates that they have rather specialized habitats, and constitute a group which should be investigated from a pathogenic viewpoint.

The various species of *Cephalosporium* have been regarded as accessory perhaps symbiotic saprophytes. Hartmann ('26) found *C. niveolanosum* associated with *Trichophyton gypseum* and left the question open. So great a number have been secured from superficial lesions that although animal inoculations may be negative (perhaps due to methods of inoculation) a parasitic nature is certainly suggested. The author has found species of *Cephalosporium* from superficial lesions of the glabrous skin especially. Various species of *Penicillium* and *Aspergillus* are even more common. *Alternaria, Sarcina lutea,* and *Torulopsis mucilaginosa* are likewise frequently found. Some species of *Cephalosporium* are also reported as saprophytic in nature on soil or humus. None of these were studied; but an organism treated by Buchanan ('11) as *Cephalosporium Pammelii* appears different from the skin group, at least in the possession of multiseptate conidia, whereas only uniseptate ones are found in the group here investigated. Species of *Cephalosporium* have also been found on insects (Petch, '28) and as causative agents in various plant diseases. In the Dutch elm disease it is only a stage of an organism, of which the perfect stage is *Ceratostomella Ulmi* of the Sphaeriales. Reddy and Holbert ('24) gave a good description of the effects of *C. Acremonium* Corda emended Fresenius, upon corn, causing the black bundle disease. In all these cases it is the transporting system which is attacked. The habitat would certainly indicate parasitic tendencies which might as well be developed in animal tissues as in those of the plant.

B. OCCURRENCE OF GRANULES IN CULTURE

One strange cultural condition certainly deserves further mention. On blood serum *C. rubrobrunneum,* after several weeks, produced numerous small white spherical bodies several mm. in diameter. Under low power they appeared surrounded by a fine felt of white to pale pink mycelium (pl. 6, figs. 15–16). These proved to be very hard and when crushed revealed a

white chalky interior. Under higher power numerous linear crystals radially arranged were seen. These crystals did not stain with lacto-phenol and cotton blue, nor with glycerine-eosin, and there was no definite cellular mass visible, although there were some irregularly shaped stainable masses. The latter were suggestive of the granular bodies occurring sometimes in the animal body, in fact quite definitely characteristic of certain diseases. Of late such have been noted more frequently upon artificial culture (Area Leão and Lobo, '34).

Two recent papers (Almeida, '34, and Weidman, '32) gave rather fully the history of granular occurrences. These varied in the host from the capsules of bacteria, *Saccharomyces hominis* and *Cryptococcus histolyticus,* to the radiating structures of purely membranal or cellular nature, as in *Aspergillus,* and the definite extra-cellular grains produced in *Actinomyces,* Madura foot, and the like. They have been interpreted as a reaction to the host comparable to distortions of haustoria, as living structures capable of absorbing food, etc., or as mere dead extensions of the membrane. They are compared to the "Hülle" cells first mentioned by Eidam ('83) in species of *Aspergillus*—all strains of the *A. nidulans* group and some strains of *A. terreus, A. flavipes,* and *A. ustus.* These cells develop from older mycelia as smaller branches, and the terminal and subterminal cells become surrounded by a very thick mucilaginous wall, comparable to that of *Saccharomyces hominis* produced in the host. The other structures have been evoked upon organic media frequently—a fact which eliminates a vital cause and probably puts such formations on a chemical basis. Weidman ('32) compared these incrustations to the similar mechanism in the "Dauercyst" of protozoa, i. e., that the capsule (especially) is protective against adversity and preparatory to reproduction, as likewise the asci in fungi are reproductive cells whose development is stimulated by unfavorable conditions. He does soften this rather far-fetched homology of reproduction and adverse conditions (although such was probably the primitive state) by saying that possibly reproduction could be absolutely separated from environmental factors. He thought that adverse conditions might also be assumed for

fungi in the comparatively anaerobic and warm environment of the mammalian tissue.

Incrustations of the type found by the author are extramatrical, of the "calcareous incrusting substance" type in Weidman's classification. Their origin and purpose are poorly comprehended, but they are probably reminiscent of pathological conditions in the host. Various solvents (concentrated acids of sulphuric, nitric, and hydrochloric) were tried on these crystalline masses which it was conjectured might be calcium oxalate. The most rapid action occurred in hydrochloric acid, releasing numerous square-oblong flat crystals and some loose cubical arrangements of about sixteen small ones; and the remainder of the ball appeared segmented with fine long crystals which protruded radially. In nitric acid there was much bubbling, and the balls assumed a rougher appearance under the microscope but dissolution was very slow. The bubbles were colorless, of a peculiar hexagonal outline, and their heavy part contained small crystalline fragments. In sulphuric acid there were crystals similar to those in the hydrochloric acid, and some hollow ones. It could not be noted if the ball were actually diminishing in mass, but the liquid around it seemed of a deeper yellow than the normal acid, perhaps indicating solution.

VII. Taxonomy and Morphology

The general classification of this group has been given in a previous section, and only the individual members will be discussed here. Due to the temporary nature of this group, much changing of nomenclature is both unnecessary and confusing. A couple of very obvious errors are corrected, however, i. e., in regard to the former *Gliocladium roseum* and *Clonostachys spectabilis*. The new organism isolated by the author is described as *Cephalosporium symbioticum*. Descriptions are based mainly upon giant colonies grown upon glycerine agar. The names of the colors are according to Ridgway ('12).

Allescheria Sacc. & Syd. in Sacc. Syll. Fung. **14**: 464. 1899. It was thought that if this form were included with a known

fertile stage more light might be shed upon the significance of the conidiophore. Unfortunately, the entire life cycle was not recalled and a complete cytological study was impossible. The conidiophores are poorly developed and resemble somewhat those of *Cephalosporium;* but the dimorphism of conidia is a constant feature, and the soft downy colony is quite distinct from the smooth rope-like one of *Cephalosporium;* so that it is doubtful if even the spore-ball stage should be included in that genus. Type: *Eurotiopsis Gayoni* Costantin.

1. ALLESCHERIA BOYDII Shear apud Boyd and Crutchfield, Am. Jour. Trop. Med. **1**: 258–268. 1921; Mycologia **14**: 239–243. 1922. Pl. 1, figs. 18–28.

Colony: pale gray velvety, of short filaments, sometimes becoming greenish in spots, or with much longer pile and approaching white, cerebriform and growing into medium at edges; reverse slightly hardened and wrinkled; growth relatively slow.

Conidiophores: simple; phialides on aerial mycelia, 3–20 μ long, septate or not, cutting off oblong conidia which adhere irregularly; spores with two oil droplets, ovoid, capable of immediate germination, 8×5.5 μ, heavy-walled spores occasionally preceding hyaline ones, the former characteristic of single-spored phialides of coremia, 15.5×4 μ; terminal chlamydospores 8.5×11 μ.

Habitat: isolated from a case of granular mycetoma in a negro. Culture of Shear, from Baarn.

CEPHALOSPORIUM Corda, Icon. Fung. **3**: 11. 1839.

Colony: smooth, much corrugated, rope-like, forming a consistent layer, slightly adherent.

Conidiophores: simple or with few short lateral branches along cords of hyphae; hyphae repent, phialides long and slender, only slightly narrower at tips than at base, bearing spores in slime balls; coremia frequent on certain media; chlamydospores present. Type: *C. Acremonium* Corda.

The six species here studied form quite a homogenous group. *Cephalosporium symbioticum* and *C. Serrae* vary most widely,

the former including yeast-like growth and becoming fuscous with chlamydospores, the latter becoming pubescent and also dark with chlamydospores.

KEY TO SPECIES OF CEPHALOSPORIUM

1. Producing some yeast-like growth on solid culture, apricot-buff, becoming fuscous ..... *C. symbioticum*
1. Not found to produce yeast-like growth in culture 2
2. Colony white with a rough dry surface, eventually becoming fuscous due to chlamydospores ..... *C. Serrae*
2. Colony not becoming fuscous 3
3. Colony moist, rope-like, turbinate 4
3. Colony dry, often with a bloom, irregularly dissected 5
4. Colony ivory to pale pink; conidiophores simple; spores 6 × 3 μ ..*C. niveolanosum*
4. Colony soon becoming deep orange; conidiophores simple or branched; spores 2–4 × 1.5 μ ..... *C. kiliense*
5. Surface of colony smooth, cerebriform, seashell pink; spores 7 × 1.5 μ ..... *C. rubrobrunneum*
5. Surface of colony short-pubescent, slightly convolute, white to salmon-buff; spores 4–5.5 × 2.5–3 μ ..... *C. Stuehmeri*

1. CEPHALOSPORIUM RUBROBRUNNEUM Nannizzi, Tratt. Micopat. Umana [Pollacci] **4**: 455. 1934. Pl. 2, figs. 22–25.

C. rubrobrunneum cerebriforme Hartmanni Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928.

Colony: at first an irregular small dry pink heap in center, spreading irregularly for about a cm., then flat, about 1 mm. thick, moderately dry, whitish pubescent at edges, center of coremia becoming frosted, seashell pink, very cerebriform, edges abrupt; little guttational water; reverse corrugated and consistent, pale orange, little color diffusing into medium; no coremial balls.

Conidiophores: borne on ropes of numerous hyphae up to 11 μ in diam., surrounded by thick mucus, spreading from both sides, non-septate, up to 30 × 2–3 μ, producing spores in balls 5–15 μ in diam., or irregularly clinging along sides of phialides in packets; spores oblong-ellipsoid, 7 × 1.5 μ, or almost spherical, 2–2.5 μ, sometimes slipping past each other at only a slight angle and forming a column; terminal chlamydospores on wort agar, 17 × 14 μ, globular crystalline bodies on serum (see pl. 6, fig. 9, and discussion p. 35).

Habitat: isolated from a case of dermatitis in Frankfort, a.M., Germany. Culture of Pollacci, from Baarn.

2. CEPHALOSPORIUM STUEHMERI Schmidt & Beyma in Beyma, Centralbl. f. Bakt. I, **130**: 102–105, *3 figs.* 1933. Pl. 2, figs. 17–21.

Colony: white to a pale pink, slightly pubescent above, little aerial growth, small white balls 1 to several mm. in diam. arising on elevated spots of colony in cultures 7–10 days old, about as thick as heavy paper; guttational water; reverse corrugated, pale orange, as is also adjacent medium; small coremia on Sabouraud's medium, and on carrot plugs a white pustular tallowy growth.

Conidiophores: unbranched, usually one to several septate, borne in a row along both sides of a straight hypha; sheaths of hyphae often with some strands protruding and bearing spores; phialides 35 × 1.5 μ; cephalosporia 10–15 μ; conidia ellipsoid, 4–5.5 × 2.5–3 μ, swelling slightly on germination, sprouting at one point and giving a mycelium that becomes early septate; vegetative cells 10 × 2 μ, uninucleate; submerged terminal chlamydospores 6.5–7 μ in diam., intercalary ones 7 μ.

Habitat: eczematoid infection of skin, Germany. Culture of Stuehmer, Baarn.

3. CEPHALOSPORIUM KILIENSE[3] (Gruetz) Hartmann, Derm. Woch. **82**: 569. 1926. Pl. 2, figs. 26–31.

Acremonium kiliense Gruetz, Derm. Woch. **80**: 765–774. 1925.

C. asteroides griseum Gruetzii Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928.

C. Acremonium Pollacci & Nannizzi, I Miceti Pat. Uomo Anim. **9**: No. 81. 1930. Non Corda, 1839.

Colony: a few mm. thick, bittersweet-orange, finely corrugated, consisting of a number of small irregularly arranged strands, aerial growth slight, flat, and parallel; mycelium extending up on glass and consisting mainly of relatively thick cords upon which tiny cephalosporia can be seen with a lens;

[3] See Dodge, Med. Myc. p. 828. 1935.

much guttational water; reverse slightly irregular, lighter-colored than upper surface; on Sabouraud's dextrose agar a faint white surface powder; on carrot plug an orange hairy coremial growth.

Conidiophores: along both sides of main stalk, especially clustered at ends and upon lateral branches where compound phialides are often borne in whorls of four or more, forming cephalosporia up to 14 μ in diam. in the air, or irregular non-slimy packets; phialides slightly tapering, 2–43 × 2.5 μ; conidia oblong-ellipsoid, hyaline, 2–4 × 1.5 μ; terminal chlamydospores 4–7 × 2–5 μ, intercalary ones 5 μ in diam.

Habitat: gummata and ulcers in man. Culture of Pollacci (?), from Baarn.

4. Cephalosporium Serrae Maffei, Atti Ist. Bot. R. Univ. Pavia IV, 1: 183–198, *9 figs.* 1930. Pl. 2, figs. 1–11.

Colony: about 5 mm. thick, corrugated, at first white then sea-shell pink, in about ten days turning fuscous, aerial growth rough and coarse wooly, of short projections several mm. high, heaped, dry and partially wrinkled; a bit of fine growth on glass; no guttational water; reverse wrinkled and black; no color diffusing into medium; on carrot plug an olive-buff pustular glabrous growth with a few pink coremia.

Conidiophores: borne laterally, simple or once-seriate, of 3–5 phialides; main stalk 42 μ long; phialides somewhat tapering, 29 × 2.5 μ; cephalosporia 7.5 μ in diam.; conidia ellipsoid, 3–4 × 1.5 μ, sometimes not in a ball; chlamydospores 7.5 × 5 μ and 10 × 3.5 μ, terminally or on strands of upright coremia.

Habitat: keratomycosis of the eye. Culture of Pollacci, from Baarn.

5. Cephalosporium niveolanosum Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928. Pl. 2, figs. 12–16.

Colony: at first elevated, ivory-white, pasty, 5 mm. in diam., becoming a slightly pink, glistening, dry heap, edges flat with radial striations arranged turbinately (in the dark remaining ivory-white but becoming pink upon exposure to daylight); short delicate coremia at times; reverse convex from medium.

Conidiophores: simple, rare, septate or not; spores ellipsoid with two oil droplets, 6 × 3 μ; hyphal cells 20 × 2.5 μ; intercalary submerged chlamydospores 6.5 μ in diam., terminal thinner-walled ones, 15 μ.

Habitat: isolated from a case of dermatitis in Leipzig. Culture of Benedek, from Baarn.

6. CEPHALOSPORIUM **symbioticum**[4] Pinkerton, n. sp.

Pl. 2, figs. 32–38, 45–46.

Colony: apricot-buff, on wort agar sometimes becoming drab-green, with smooth surface except for small coremia, convoluted, growing into medium below; on liquid media producing a ring or pellicle, decolorizing litmus, coagulating milk, liquefying gelatine, no gas in sugars, producing acid in dextrose, d-levulose, raffinose, maltose, and sucrose, alkali in amygdalin, arabinose, d-galactose, lactose, and dextrin.

Within medium colony forms pseudomycelium of cells 4.0 × 2.5 μ, cephalosporia 8 μ in diam. at the apex of phialides, the latter 5 μ in length; aerial thallus a true mycelium, cells 8–10 × 2 μ; phialides 2–5 μ in diam.; spores 4–5 × 2.5 μ, some germinating in place. Mature colonies becoming fuscous with many terminal and intercalary chlamydospores 4–5 μ in diam.

Habitat: isolated from a severe case of dermatitis at the Barnard Free Skin and Cancer Hospital. It was intimately associated with *Torulopsis mucilaginosa* from which it was separated only by extreme dilution cultures. The subgenus *Cephaloblaston* of Ciferri ('32), typified by *C. pseudofermentum*, would also encompass this species.

[4] CEPHALOSPORIUM **symbioticum**, sp. nov.

Colonia: "apricot-buff" aliquando "drab-green," in medio musto, superficie laevi praeter coremia parvo, convoluta, in media subter succrescens; in mediis liquidis annulum pelliculumve efficiens, litmo decolorans, lactem coagulans, gelatinam liquefaciens, in saccharis non effervescans; acidum in "dextrose, d-levulose, raffinose, maltose, sucrose" faciens, alcalinum in "amygdalin, arabinose, d-galactose, lactose, dextrin."

Colonia mycelium falsum intra medium formans, cellulis 4.0 × 2.5 μ, cephalosporiis 8 μ in diametro apice phialae 5 μ in longitudine; thallus aerius mycelium verum, cellulis 8–10 × 2 μ; phialae 2–5 μ in diametro; sporae 4–5 × 2.5 μ, aliquibus in situ germinantibus. In coloniis maturis fuscescentibus chlamydosporae multae terminales aut intercalares, 4–5 μ in diametro.

Case History.—The patient, a white female 15 years of age, first reported to the Barnard Free Skin and Cancer Hospital on April 2, 1934. She had a severe dermatitic eruption occurring in patches upon her hands, especially the fingers, and upon the forearms. The case was diagnosed as "dermatitis contact." Trichophytin tests were given and crude coal-tar salves advised. Various patent medicines previously used by the patient complicated the irritation. By August 31, 1934, there was a marked improvement, but on September 20, the condition was worse and X-ray treatments were given. On October 2, the hands showed many new vesicles and bullae and ruptured blisters which left raw spots. The condition was then diagnosed as a combination of tinea and *Staphylococcus* infection. She also suffered from acne and reported a history of scabies. In addition to X-ray treatments, a tincture of xeroform was recommended. The patient was last heard from on October 24, 1934.

Skin cultures included numerous organisms of which *Staphylococcus*, *Torulopsis mucilaginosa*, *Cephalosporium symbioticum*, and a black filamentous form (unidentified) were dominant. The *Cephalosporium* was non-pathogenic to white mice.

CLONOSTACHYS[5] Corda, Pracht-Flora, p. 31. 1839.

Creeping hyphae; conidiophores simple or branched; phialides four or more branched, verticillate; spores adhering in cephalosporia. Type: *C. Araucaria* Corda.

1. CLONOSTACHYS ARAUCARIA Corda, Pracht-Flora, p. 31. 1839. Pl. 5, figs. 14–26.

Colony: white-cottony, soon turning pale flesh to seashell pink, powdery, rapidly growing; coarse white-pink coremia in older cultures; mycelium extending up glass for several mm., bearing cephalosporia; much guttational water; reverse wrinkled. Species differs superficially from the former *Gliocladium roseum* in pinker color, slower growth, less extensive coremia formation, and producing acid in maltose.

Conidiophores: polymorphic, some of the characteristic of four-branched type; asymmetrical penicillate fructifications

[5] The investigation of the type species and varieties permits enlargement upon Corda's original limited description. Unfortunately, the author is unfamiliar with the other species usually included here, but because of the limited generic description it is doubtful if they form a homogeneous group. The type, which has been inadequately characterized, does seem sufficiently different to justify its position in a genus intermediate between *Cephalosporium* and *Gliocladium*. *Clonostachys spectabilis*, which was studied only briefly due to an early demise, obviously is far removed and certainly suggests *Botrytis*. Presumably Oudemans ('86) compared the botryoid spores to the lateral protrusions sometimes found on *C. Araucaria* and its varieties, which are not spores.

formed on reversed wort plates with terminal clusters of six phialides and laterals of three to six, phialides 10 × 2 μ, flask-shaped and bent outwards, conidia spherical to ellipsoid, 3 × 1–2 μ; cephalosporia borne along the main cord, singly or in clusters of three to six, phialides slightly flask-shaped, 17–33 × 1.5–4 μ; cephalosporia 13 μ, conidia 5.5–6.5 × 2.5–3 μ; hyphae warty, especially near cephalosporia; chlamydospores rare, on corn meal agar in terminal series, 13 × 10 μ; or on lactose broth intercalary, 10 × 12.5 μ; hyphal cells 13 × 3 μ.

Habitat: "In cortice ramulorum arborum in Germania, Brittania" (Corda). Culture of Wakefield, from Baarn.

2. CLONOSTACHYS ARAUCARIA var. ROSEA Preuss, Linnaea **25**: 727. 1852. Pl. 4, figs. 27–31.

Colony: salmon-pink almost at first, mottled with white, floccose, of strands several mm. long, heaped in center and irregularly wrinkled, coarsely flocculent at edges; much guttational water; reverse wrinkled. Variety differs from the former *Gliocladium roseum* and the species in the salmon tint, intermediate speed of growth, and is more wrinkled than either.

Conidiophores: mostly typical of four phialides as illustrated by Corda, the spores from several phialides sometimes forming confluent slime balls; cephalophores up to 100 μ; phialides non-septate, 17–40 × 6 μ; cephalosporia 21 μ in diam.; conidia 4 × 5 μ; chlamydospores terminal and intercalary, 3.5 × 10 μ.

Habitat: "In ramulis, in piris et pomis immaturis dejectis, frequens, Hoyerswerda" (Preuss). Culture of Shapovalov, from Baarn.

3. CLONOSTACHYS ARAUCARIA var. **confusa** Pinkerton, n. var. Pl. 4, figs. 15–26.

Penicillium roseum Link (?), Ges. Naturforsch. Fr. Berlin, Mag. 7: 37. 1816.

Gliocladium roseum Bainier, Bull. Soc. Myc. France **23**: 111–112, *pl. XV, figs. 1–6.* 1907. Nec *Clonostachys Araucaria* var. *rosea* Preuss.

Colony: pale ochraceous salmon, slightly mottled with

white, short-cottony and mealy on top, growing up tube very little, slightly adherent; on inverted Petri plate of wort agar producing white feathery extensions measuring up to 1 cm. long; much guttational water; reverse much wrinkled.

Conidiophores: polymorphic; penicillate conidiophores asymmetrical with branches terminating in whorls of three to four, penicillus 85 μ high, main lateral segments 45 μ, phialides 8–10 × 1.5–2 μ, flask-shaped, mature conidia spherical to slightly falcate, 5 μ, swelling on germination to 8–10 μ, growing from one or both poles; spore balls from single phialide along main hyphae or rope, or usually in whorls of three, borne laterally or terminally, phialides 30–40 × 3–4 μ, warty, straight or slightly narrower at top, adjacent balls sometimes becoming confluent and reaching 10–30 μ in diam., conidia ellipsoid, smooth, hyaline, pink in mass, 6–7 × 4–5.5 μ; chlamydospores rarely formed but found terminally on submerged hyphae on Raulin's agar, 4.5 μ in diam., also on inverted wort plates, intercalary ones 14 × 20 μ.

Habitat: "In caule solani tuberosi exsiccati. Misit Dr. Nees ab Esenbeck." (Link); "du carton pourri" (Bainier). Culture from Thom No. 454–4640.428, from Kral in Prague, Bohemia.

Bainier stated that this form resembled *Acrostalagmus roseus* of the Verticillieae except that the precedence of penicillate over verticillate series was reversed in *Gliocladium*. More probably the variations are dependent upon nutrient relations, especially moisture conditions. On lactose broth hanging drops, the spore balls are most luxuriant, as are also the warts. Complex penicillate fructifications are also present, however, and in fact precede the simpler cephalosporial stage both in time and on the main trunk. The spores on the penicillus cling together only loosely in irregular chains as seen on slide cultures, and are quickly disseminated in water. Conspicuous deliquescence of the penicillate heads, as occurs in *G. deliquescens*, was not noted. The fact that in crossing this form is antagonistic to the species, whereas the rest are all indifferent, might indicate a close relationship of a lethal nature.

4. CLONOSTACHYS SPECTABILIS (Harz) Oudem. & Sacc. Ned. Kruidk. Arch. II, **4**: 539. 1886. Pl. 5, figs. 1–7.

Botrytis spectabilis Harz, Bull. Soc. Imp. Nat. Moscow **44**: 88–147. 1871.

Original colony gray-white and fuzzy, turning black due to production of numerous conidia.

Conidiophores compound, dichotomously branched and bearing spores on short stalks laterally along the phialides; spores spherical, 6.5–7 μ in diam.; chlamydospores 6.5–10 μ in diam.

Habitat: "sur la tannée dans les serres du jardin botanique d' Amsterdam" (Balen). Culture of Shear, from Baarn.

GLIOCLADIUM[6] Corda, Icon. Fung. **4**: 30–31. 1840.

Conidiophores erect, septate, penicillately branching above, branches and branchlets septate, appressed, forming a solitary gelatinous head; conidia unicellular, borne upon the tips of branchlets and held together by mucilaginous substance in a dense mass, those on adjacent phialides fusing. Type: *G. penicilloides* Corda.

1. GLIOCLADIUM FIMBRIATUM Gilman & Abbott, Iowa State Coll. Jour. Sci. **1**: 304, *fig. 38*. 1927. Pl. 3, figs. 33–41.

Colony: at first white, fuzzy, nearly circular, very thin, moist, translucent with parallel wrinkles, finally antique green, mottled with white, dry, floccose, faintly zonate and radially striate, broad masses of white hyphae growing up tube several cm.; reverse radially corrugate.

[6] The inclusion of the former *Gliocladium roseum* in *Clonostachys* might possibly also justify lumping *Gliocladium* in that genus whose publication preceded it by one year. However, the three sections of *Gliocladium* mentioned by Thom ('30)—the rose, white, and green series—seem quite distinct culturally and morphologically. There is one common character to the two genera, i. e., the lack of disjunctors and presence of cephalosporia, which in view of the heterogenous mass included under *Penicillium* might make such a segregation logical.

Gliocladium penicilloides resembles *Clonostachys Araucaria* in a rather similar zonate growth and exudation of yellow color into medium, but differs in the thin-walled auxiliary cells (akinetes). *G. Vermoesenii* is quite distinct in its colonial characters and the spore type which approaches *Penicillium* in its rigidness, but still lacks disjunctors. *G. deliquescens* and *G. fimbriatum* are very different from each other as well as the others in colonial characters but have akinetes. Therefore, due to insufficient knowledge of the *Gliocladium* and *Clonostachys* segregates as a whole, they will not be combined here.

Conidiophores: homogeneous, borne laterally and terminally in a wide range of heights up to 300 μ, more or less symmetrical in two series, basal cell 14.5 × 5.3 μ; first series 6.5 × 4 μ; phialides very turgid and asymmetrically flask-shaped, 8.5 × 4.5 μ; spores ellipsoid, 4.5 × 3.3 μ, forming irregular spore balls up to 16.5 μ in diameter, dry and only slightly mucoid; akinetes terminal and intercalary, frequent, 11–13 × 8.5–10 μ, containing oil droplets up to 1.3 μ, these soluble in chloroform leaving striations; chlamydospores intercalary, 9–9.5 × 8 μ.

Habitat: in soil, Iowa and Louisiana. Culture of Gilman & Abbott, from Thom No. 459–4894.20.

2. Gliocladium deliquescens Sopp, Monogr. pp. 89–93, *taf. I, fig. 1–6.* 1912. Pl. 3, figs. 20–32.

Colony: at first colorless, of mainly submerged hyphae, quickly becoming thin and white-floccose over whole surface, then meadow-green and slimy, progressing slowly centripetally from edge; much guttational water; very rapid growth of hyaline semi-submerged radiating hyphae.

Conidiophores: of two types—penicillate and simple cephalosporial; penicilli at first white but spores become green and aggregate in a heavy slime, typically tri-seriate and each series trichotomous; brush 45 × 40 μ; first series 17.5–24 × 4 μ; second series 9–11 × 4 μ; phialides 6–9 × 2–2.5 μ, spores smooth, spherical to ellipsoid, 3.5 × 2.5 μ, germinating spores swelling up to 6.5–9 μ, sprouting in one or more places; spore balls up to 30 μ; cephalosporia up to 60 μ, circular with thick matrix of slime, cephalolides 40 × 6 μ; conidia 2 μ in diam.; akinetes 7.5–10 × 6.5–8.5 μ, terminal or intercalary; pseudoterminal ovoid cells 14 × 11.5 μ; chlamydospores 6 × 7.5 μ.

Habitat: on *Daedalea unicolor* in Norway. Culture of Thom No. 457–4894.17.

3. Gliocladium Vermoeseni (Biourge) Thom, The Penicillia, p. 502. 1930. Pl. 4, figs. 1–14.

Penicillium Vermoeseni Biourge, La Cellule **33**: 230. 1923.

Colony: pale vinaceous, rapidly growing, irregularly powdery and with thin long hairs; much guttational water; reverse homogeneous and when viewed through appears splotchy.

Conidiophores: usually penicillate, 45 μ long from first branch, decidedly asymmetric, poly-seriate, final series trichotomous; phialides up to 10 × 1.5–3.5 μ; spores spherical, 2.5 μ; cephalolides 40 μ and more, cephalosporia 20–25 μ, cephalospores ellipsoid, 5–6.5 × 4 μ; intercalary chlamydospores 13 × 15.5 μ, terminal 5 × 11 μ; akinetes 15 μ in diam.; arthrospores 10.5 × 5 μ; oidia 3 × 5.5 μ, rare.

Habitat: parasitic or semi-parasitic on certain species of *Areca*. Culture of Westerdijk, from Thom No. 451–5090.4a.

4. GLIOCLADIUM PENICILLOIDES[7] Corda, Icon. Fung. 4: 31, *taf. 7. figs. 88–89.* 1840. Pl. 3, figs. 1–19.

Colony: at first chalky-white, of short regularly centrifugally appressed hairs, faintly and scarcely striate, within a month very dry and tan to light pink. On glycerine agar sometimes producing a lemon-yellow pigment which diffuses into the medium; growth relatively slow and consistent.

Conidiophores: asymmetric, often borne as more or less short laterals along main branch, up to 114 μ long, septate; phialides 17 × 3 μ, slightly flask-shaped, borne in threes, occasionally fours or more, of which one is larger; lowest branches alternate, usually not more than three main divisions; spores ellipsoid, 5.5–6.5 × 3 μ, swelling before germination to 8 × 5 μ; single branched phialides, septate or not, 35.5 × 2.5 μ, bearing cephalosporia 14 μ in diam.; spores 4 × 2.5 μ; vegetative cells 3.5–5 μ in diam., racquet-shaped, consisting of several large vacuoles; akinetes 10 μ in diam., full of oily reserves and capable of almost immediate germination.

Habitat: on rotting *Thelephora*. Culture of Thom No. 453–5113.

SCOPULARIOPSIS Bainier, Bull. Soc. Myc. France **23**: 98. 1907.
Type: *Penicillium brevicaule* Saccardo.

[7] Winter ('87) suggested that probably *Eurotium insigne* Winter was the ascosporic form of this species. Matruchot ('95) found perithecia of the Plectascales type, but through the unique method of ascospore germination distinguished it from *E. insigne;* however, he offered no other nomenclatorial suggestion. Theoretically, the species should be removed to the perfect classification, but for the present such shall not be attempted.

1. SCOPULARIOPSIS BREVICAULIS (Sacc.) Bainier, Bull. Soc. Myc. France **23**: 99–103, *pl. 11, figs. 1–6*. 1907. Pl. 5, figs. 8–13.

Penicillium brevicaule Sacc. Fung. Ital. *taf. 893*. 1881.

White flocculent growth upon glycerine agar.

Conidiophores simple or few-branched; phialides 8 × 2–3 μ; spores 5 μ in diam. with disjunctors between, adhering in chains indefinitely; no mucus; no chlamydospores noted.

Habitat: one strain reported as pathogenic by Dr. D. J. Davis of Chicago. Culture of Thom No. 4858.

This species was studied only very superficially as living material, in an attempt to correlate the presence of disjunctors and no slime with the lack of disjunctors and mucus, also to note methods of phialide mechanism.

TORULOPSIS Berlese, Giorn. Viticoltura ed Enologia p. 54. 1894.

Type: *T. rosea* Berlese.

TORULOPSIS MUCILAGINOSA (Jörgensen) Ciferri & Redaelli, Atti Bot. Ist. R. Univ. Pavia III, **2**: 256. 1925.

Pl. 2, figs. 39–44, 47.

Torula mucilaginosa Jörgensen, Die Mikroorg. d. Gärungsindustrie. ed. 5, 402. 1909.

Rhodotorula mucilaginosa Harrison, Trans. Roy. Soc. Canada **22**: 187. 1928.

Cryptococcus rubrorugosus Castellani, Arch. of Derm. & Syphilol. **16**: 403. 1927.

Rhodotorula mucilaginosa race *rubrorugosa* Lodder, Anaskosporogenen Hefen **1**: 104–105. 1934.

Colony: Carnelian red to scarlet, moist and smooth, pasty, rapidly growing upon wort and maltose agar, with elliptical cells showing bipolar budding and a pair of oil droplets in mature cells; on nitrate medium, following the sparse red surface growth, a submerged irregular hyaline one of pseudomycelial cells and small asymmetrical cephalosporia up to 15 μ sometimes occurring, spores 2.5 × 5 μ; in malt broth a ring, some red basal proliferation and slight pseudomycelial growth throughout liquid; slight growth in ethyl alcohol; ring in grape juice but no fermentation, no liquefaction of gelatin, milk clotted; no

gas in sugar solutions; acid produced in sucrose, d-levulose, maltose and dextrose, alkali in arabinose, amygdalin, d-galactose, lactose, raffinose and dextrin.

Habitat: originally isolated as a laboratory contaminant; Castellani reported it from scrapings in the axilla; and it was secured by the author from skin scrapings in a severe dermatomycosis.

VIII. Summary

1. The term cephalophore has been applied to a conidial fructification wherein the spores are held in a spherical mass by mucilage. These spores are found to be produced endogenously by cleavage following exudation of liquid which forms the matrix of the spore ball. Terminal granules seem to be kinetic centers associated with exudation and spore discharge. The phialides are thought to be uninucleate as are also the spores.

2. Accessory spore forms include chlamydospores in all cases, associated both with the aerial and submerged mycelium, oidia infrequently, and thin-walled spores in some, which have been called akinetes after the algological terminology.

3. An attempt has been made to correlate comparative cultural data regarding color, macroscopic and microscopic characters. It is believed that such factors as hydrogen-ion concentration, surface tension, oxygenation, and the nature of the nutrients are especially important in producing favorable or unfavorable conditions for the organism. Good growth is characterized by abundance of aerial mycelia with a considerable pigmentation; and poor growth, by numerous sporeforms, attenuated aerial hyphae with proportionally more reproductive bodies and a tendency towards submerged growth and exudation of pigments into the medium, and a paler color of the organism. *Torulopsis mucilaginosa* develops a pseudomycelium in the submerged state and loses its red color.

4. Mycelial pigmentation is noted in some cases to be more intense under optimal conditions; in some to be light labile. The solubilities of the pigment of *Cephalosporium kiliense* are listed, although no identification is made.

5. Polarized light indicates no structural difference between the walls of the phialides and of the mycelial cells.

6. Various unsuccessful attempts have been made to produce sexual fructifications. Theories regarding loss of sexuality are discussed, but no further contributions are suggested.

7. The clinical findings of some of the organisms in dermatomycoses are mentioned, although their pathogenicity has not been proven. The granular incrustations upon serum are described. These were found to be composed of fine needle-like crystals arranged radially and including no recognizable organic matrix but surrounded by fine mycelial filaments. The crystals were slowly soluble in the concentrated heavy acids. The theoretical significances of these structures are discussed.

8. A brief morphological description is given of all the forms studied. *Gliocladium roseum* (Link?) Bainier is described as *Clonostachys Araucaria* var. *confusa*. The position of *Gliocladium penicilloides* is discussed. The new species isolated from the dermatomycosis case at the Barnard Free Skin and Cancer Hospital is designated as *Cephalosporium symbioticum*.

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Explanation of Plate

PLATE 1

All drawings, except fig. 17, made with the aid of a camera lucida.

Fig. 1. Mature compound cephalosporium in *Clonostachys Araucaria*. Nutrient broth. × 750.

Fig. 2. Incipient phialide in *Gliocladium penicilloides*. Glycerine agar. × 750.

Figs. 3–4. Basophilic granules in phialides of *G. penicilloides*. Glycerine agar. × 1250.

Fig. 5. Exudation of mucilage in *Clonostachys Araucaria*. Nutrient broth hanging drop. × 1250.

Fig. 6. Phialide enclosed in mucilage in *C. Araucaria*. Endo's agar. × 750.

Fig. 7. Orientation of granules at tips of phialides, and cleavage vacuoles in *Gliocladium penicilloides*. Glycerine agar. × 1250.

Fig. 8. Splitting of phialide tip in *G. penicilloides*. Glycerine agar. × 1250.

Figs. 9–10. Emergence of conidia in *G. penicilloides*. Glycerine agar. × 1250.

Fig. 11. Endoconidia in *G. penicilloides*. Glycerine agar. × 1250.

Fig. 12. Twisted phialide with uninucleate endoconidia in *G. penicilloides*. Glycerine agar. × 1250.

Fig. 13. Mature uninucleate conidium of *G. penicilloides*. Glycerine agar. × 1250.

Fig. 14. Conidiophores with endoconidia, and bi-vacuolate spores in *Clonostachys Araucaria* var. *confusa*. Litmus milk. × 1250.

Fig. 15. Phialides of *Gliocladium penicilloides* from paraffin sections at 3 μ. Glycerine agar. × 1250.

Fig. 16. Constriction of spore mass during exit in *G. penicilloides*. Litmus milk. × 1000.

Fig. 17. Diagram showing successive production of conidia, from observations at the end of 10, 20, 35, 65, and 70 minutes in *Clonostachys Araucaria*. Sabouraud's dextrose agar.

Figs. 18–28. *Allescheria Boydii*.

Fig. 18. Submerged chlamydospore. Nitrate agar. × 750.

Fig. 19. Conidium. Raulin's agar. × 750.

Figs. 20–21. Stages of development of submerged cephalosporia. Sabouraud's dextrose agar. × 350.

Fig. 22. Coremium stage. Sabouraud's dextrose agar. × 1250.

Fig. 23. Submerged lateral spore. Raulin's agar. × 1250.

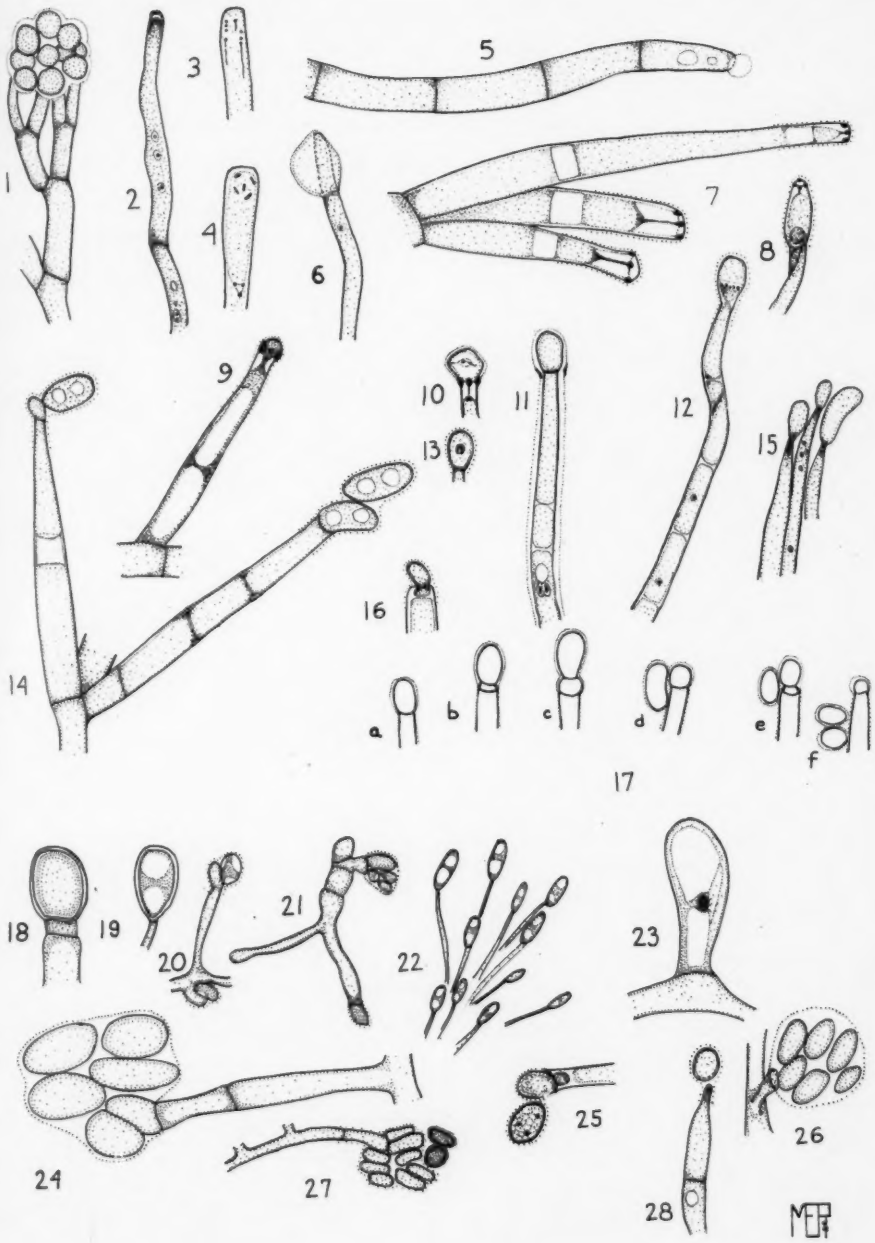
Fig. 24. Cephalosporium. Sabouraud's dextrose agar. × 1250.

Fig. 25. Mature and young spore. Sabouraud's dextrose agar. × 350.

Fig. 26. Lateral cephalosporium. Sabouraud's dextrose agar. × 1250.

Fig. 27. Young and mature spores in cephalosporium. Raulin's agar. × 350.

Fig. 28. Conidiophore. Raulin's agar. × 1250.



PINKERTON — CONIDIAL FORMATION

AYNE, BOSTON

Explanation of Plate

PLATE 2

Unless otherwise stated, all drawings were made with the aid of a camera lucida.

Figs. 1–11. *Cephalosporium Serrae.*
Fig. 1. Terminal chlamydospore. Potato agar. × 1250.
Fig. 2. Series of chlamydospores. Potato agar. × 750.
Fig. 3. Chlamydospores. Corn meal agar. × 750.
Fig. 4. Cephalosporium within medium. Raulin's agar. × 1250.
Fig. 5. Twisted phialide with endoconidia. Sabouraud's dextrose agar. × 1250.
Fig. 6. Phialide with vacuoles. Sabouraud's dextrose agar. × 1250.
Fig. 7. Dimorphic cephalosporium. Maltose agar. Not camera lucida.
Fig. 8. Diagram of fructification.
Fig. 9. Germinating chlamydospore. Nutrient broth. × 750.
Fig. 10. Mucilage drop. Nutrient broth. × 750.
Fig. 11. Packet type of cephalosporium. Nutrient broth. × 750.
Figs. 12–16. *Cephalosporium nivcolanosum.*
Fig. 12. Terminal chlamydospore. × 750.
Fig. 13. Chlamydospores. Corn meal agar. × 750.
Fig. 14. Phialide with endoconidia. Nutrient agar. × 750.
Fig. 15. Phialide producing spore. Nutrient agar. × 750.
Fig. 16. Young cephalosporium. Nutrient agar. × 750.
Figs. 17–21. *Cephalosporium Stuehmeri.*
Fig. 17. Conidiophore with endoconidium. Sabouraud's dextrose agar. × 750.
Fig. 18. Diagram of fructification.
Fig. 19. Terminal chlamydospore. Corn meal agar. × 750.
Fig. 20. Chlamydospores. Sabouraud's dextrose agar. × 350.
Fig. 21. Peculiar aerial spore. Sabouraud's dextrose agar. × 350.
Figs. 22–25. *Cephalosporium rubrobrunneum.*
Fig. 22. Origin of phialides from ropes of hyphae. Glycerine agar. × 350.
Fig. 23. Slanted spore cluster. Sabouraud's dextrose agar. × 350.
Fig. 24. Young cephalosporium. Sabouraud's dextrose agar. × 350.
Fig. 25. Uninucleate spore. Sabouraud's dextrose agar. × 350.
Figs. 26–31. *Cephalosporium kŭlense.*
Fig. 26. Diagram of fructification. Endo's agar.
Fig. 27. Cephalosporium. × 350.
Fig. 28. Packet cephalosporium. Sabouraud's dextrose agar. Not camera lucida.
Fig. 29. Cephalosporium. Sabouraud's dextrose agar. × 350.
Fig. 30. Chlamydospores. Corn meal agar. × 1250.
Fig. 31. Chlamydospores. Malt extract broth. × 1250.
Figs. 32–38. *Cephalosporium symbioticum.*
Fig. 32. Chlamydospore. Glycerine agar. × 750.
Fig. 33. Conidium. Nutrient broth. × 1250.
Fig. 34. Cephalosporia. Endo's agar. × 600.
Fig. 35. Conidia. Nutrient agar. × 1250.
Fig. 36. Young submerged growth. Sabouraud's dextrose agar. × 1250.
Fig. 37. Submerged growth on wort agar. × 1250.

EXPLANATION OF PLATE

PLATE 2 *(Continued)*

Fig. 38. Chlamydospores. Sabouraud's dextrose agar. × 1250.

Figs. 39–44. *Torulopsis mucilaginosa.*

Fig. 39. Resting cell. Malt extract broth. × 750.

Fig. 40. Bipolar budding. Malt extract broth. × 750.

Fig. 41. Submerged pseudomycelium. Nitrate agar. × 750.

Fig. 42. Pseudomycelium and irregular spore clusters. Endo's agar. × 600.

Fig. 43. Submerged cephalosporium. Nitrate agar. × 750.

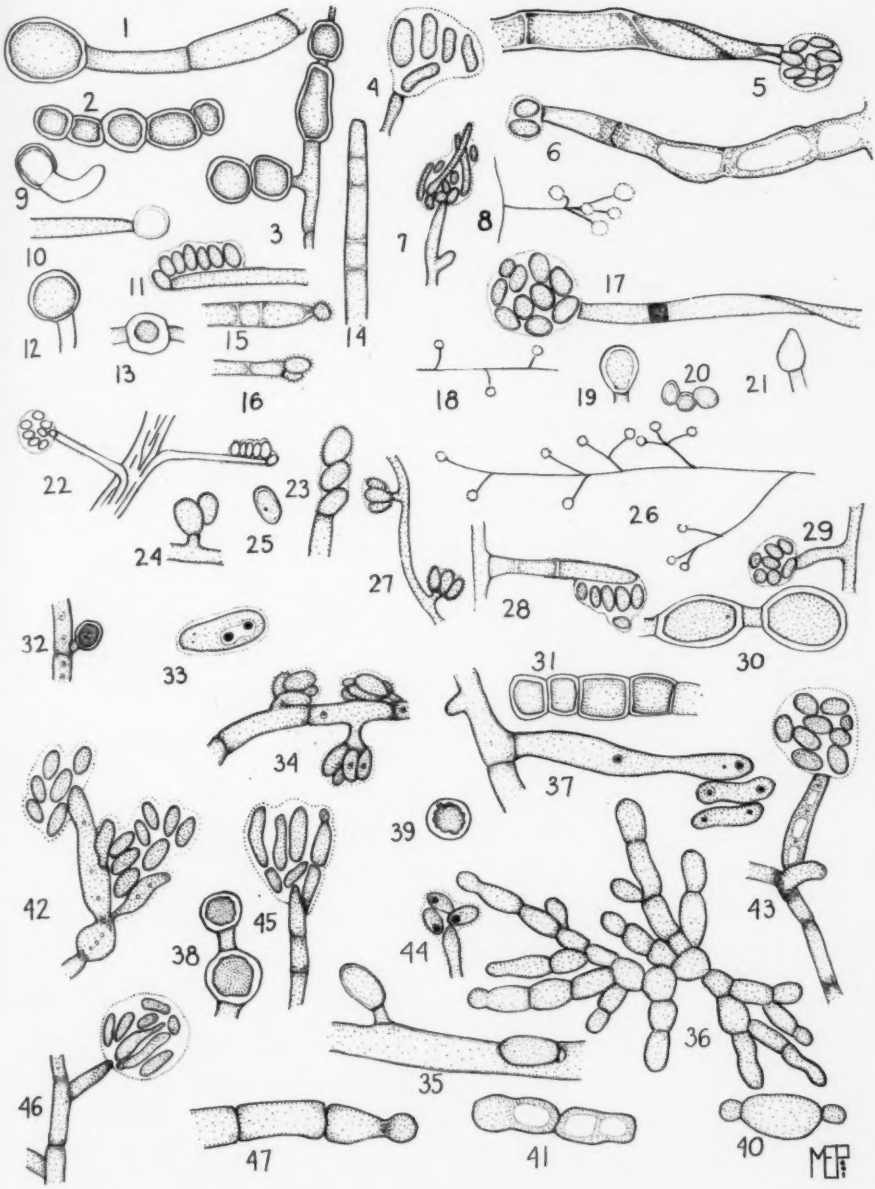
Fig. 44. Submerged sporiferous tip. Nitrate agar. × 750.

Figs. 45–46. *Cephalosporium symbioticum.*

Fig. 45. Cephalosporium with spore germinating *in situ.* Wort agar. × 1250.

Fig. 46. Cephalosporium. Wort agar. × 1250.

Fig. 47. Submerged mycelium of *Torulopsis mucilaginosa,* forming new cell by constriction. × 1250.



PINKERTON — CONIDIAL FORMATION

E, BOSTON

Explanation of Plate

PLATE 3

Drawings made with the aid of a camera lucida.

Figs. 1–19. *Gliocladium penicilloides.*

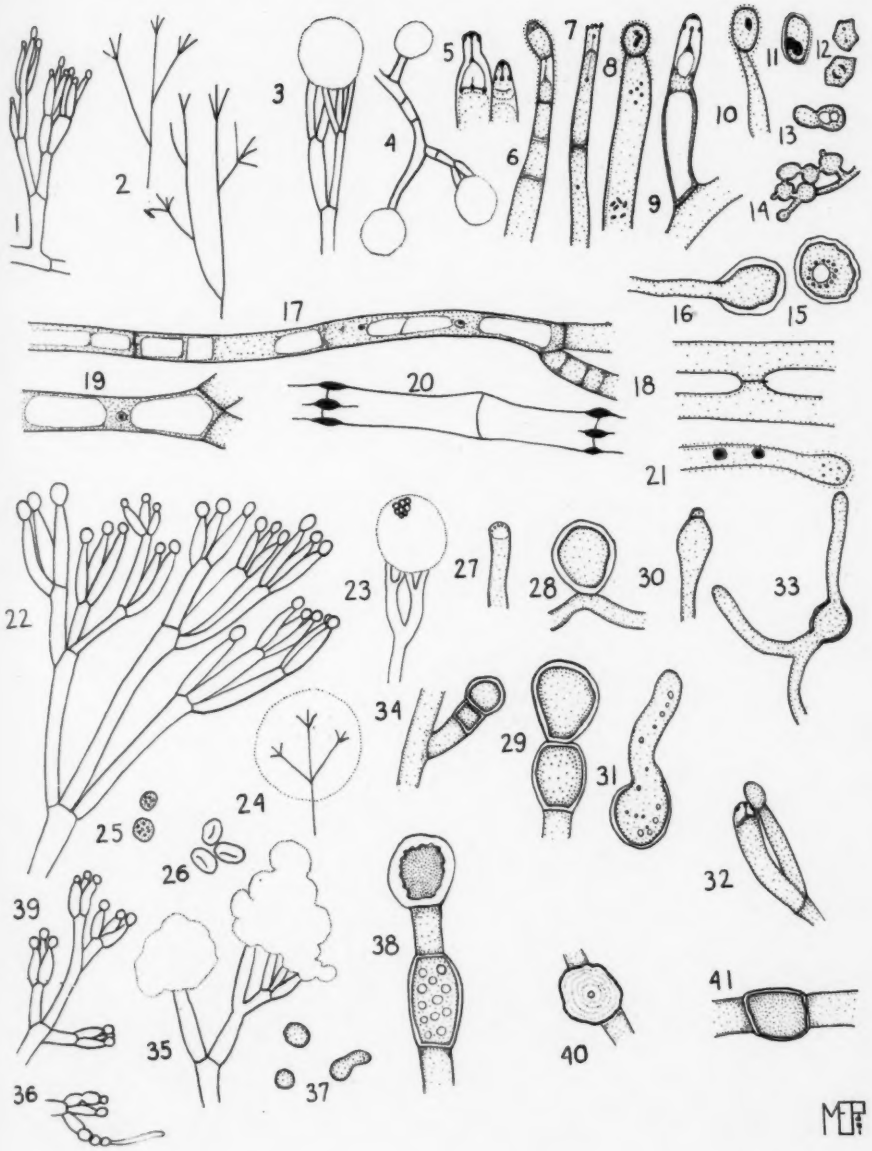
Fig. 1. Conidiophore. Glycerine agar. × 350.
Fig. 2. Diagram of fructification.
Fig. 3. Cephalosporium. Nutrient broth. × 350.
Fig. 4. Cephalosporia. Nutrient broth. × 600.
Fig. 5. Phialides with basophilic granules. Glycerine agar. × 1250.
Fig. 6. Phialide with mature spore and endoconidia. Glycerine agar. × 1250.
Fig. 7. Phialide with broken tip. Glycerine agar. × 1250.
Fig. 8. Phialide with basophilic granules. Glycerine agar. × 1250.
Fig. 9. Phialide with basal vacuole. Glycerine agar. × 1250.
Fig. 10. Conidium. Glycerine agar. × 1250.
Fig. 11. Conidia showing granular material. Glycerine agar. × 1250.
Fig. 12. Conidia. Glycerine agar. × 1250.
Fig. 13. Germinating conidium. Glycerine agar. × 750.
Fig. 14. Fusions of germ tubes of conidia. Nutrient broth. × 350.
Fig. 15. Akinete. Glycerine agar. × 750.
Fig. 16. Germinating akinete. Glycerine agar. × 750.
Fig. 17. Hypha. Glycerine agar. × 1250.
Fig. 18. Hyphal fusions. Nutrient broth. × 1250.
Fig. 19. Sub-phialide cell. Glycerine agar. × 1250.

Figs. 20–32. *Gliocladium deliquescens.*

Fig. 20. Submerged mycelial cell showing granular bodies at septa. Glycerine agar. × 1250.
Fig. 21. Tip of mycelial cell with large dancing bodies and small terminal granules. Nutrient broth. × 1250.
Fig. 22. Young conidiophore. Glycerine agar. × 1000.
Fig. 23. Cephalosporium. × 350.
Fig. 24. Diagram of cephalosporium branching structure.
Fig. 25. Conidia with chromatic material. Glycerine agar. × 1250.
Fig. 26. Conidia from smear stained with gentian violet. × 1250.
Fig. 27. Aerial hypha with granular tip. Glycerine agar. × 1250.
Fig. 28. Chlamydospore (?). Sabouraud's dextrose agar. × 800.
Fig. 29. Chlamydospores. Glycerine agar. × 1000.
Fig. 30. Aborted ascogonium (?). Glycerine agar pH 3.8. × 350.
Fig. 31. Germinating akinete. Sabouraud's dextrose agar. × 750.
Fig. 32. Phialide with terminal granules. Glycerine agar. × 1250.

Figs. 33–41. *Gliocladium fimbriatum.*

Fig. 33. Germinating conidium. Endo's agar. × 600.
Fig. 34. Chlamydospores. Sabouraud's dextrose agar. × 600.
Fig. 35. Conidiophore. Corn meal agar. × 750.
Fig. 36. Dimorphic fructification. Sabouraud's dextrose agar. × 350.
Fig. 37. Conidia. Sabouraud's dextrose agar. × 750.
Fig. 38. Akinetes. Glycerine agar. × 750.
Fig. 39. Conidiophore. Sabouraud's dextrose agar. × 350.
Fig. 40. Akinete upon addition of alcohol and chloroform, showing dissolution of oil. × 750.
Fig. 41. Intercalary chlamydospores. Corn meal agar. × 750.



PINKERTON — CONIDIAL FORMATION

Explanation of Plate

PLATE 4

Drawings made with the aid of a camera lucida.

Figs. 1–14. *Gliocladium Vermoeseni.*

Fig. 1. Submerged chlamydospores. Sabouraud's dextrose agar. × 350.

Fig. 2. Aerial chlamydospores. Raulin's agar. × 750.

Fig. 3. Endoconidial method of formation of chlamydospores. Sabouraud's dextrose agar. × 750.

Fig. 4. Arthrospores. Corn meal agar. × 750.

Fig. 5. Conidiophore. Sabouraud's dextrose agar. × 750.

Fig. 6. Mature conidium. Sabouraud's dextrose agar. × 750.

Fig. 7. Part of cephalosporium showing exterior thick granular layer of mucus and spores deformed by pressure. Sabouraud's dextrose agar. × 750.

Fig. 8. Branch of penicillate fructification. Czapek's agar. × 350.

Fig. 9. Conidiophores. Corn meal agar. × 750.

Fig. 10. Unusual conidiophore. Raulin's agar. × 750.

Fig. 11. Oidia (†). Raulin's agar. × 750.

Fig. 12. Anomalous structure. Raulin's agar. × 1250.

Fig. 13. Diagram of fructification.

Fig. 14. Submerged akinete. Raulin's agar. × 750.

Figs. 15–26. *Clonostachys Araucaria* var. *confusa.*

Fig. 15. Conidiophore. Czapek's agar. × 350.

Fig. 16. Conidiophore with warts. Czapek's agar. × 350.

Fig. 17. Short lateral cephalosporium. Sabouraud's dextrose agar. × 350.

Fig. 18. Phialides with endoconidia. Endo's agar. × 1000.

Fig. 19. Chlamydospore (†). Sabouraud's dextrose agar. × 600.

Fig. 20. Dimorphic fructification. Lactose broth. × 600.

Figs. 21–22. Copulating conidia. Litmus milk. × 1250.

Fig. 23. Mature conidium. × 750.

Fig. 24. Germinating conidium. Sabouraud's dextrose agar. × 750.

Fig. 25. Hyphal fusions. Endo's agar. × 350.

Fig. 26. Submerged chlamydospore. Raulin's agar. × 750.

Figs. 27–31. *Clonostachys Araucaria* var. *rosea.*

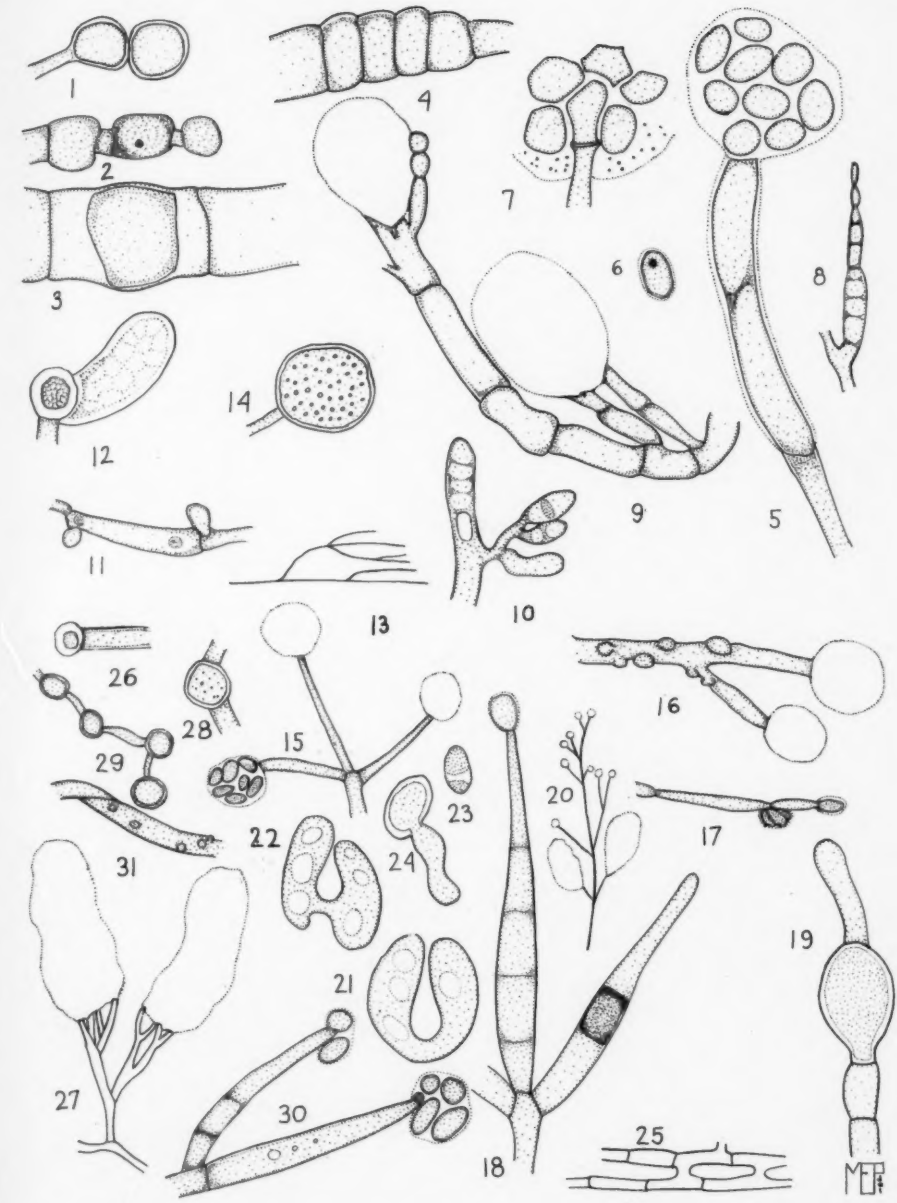
Fig. 27. Cephalosporia. Endo's agar. × 250.

Fig. 28. Chlamydospore. Corn meal agar. × 750.

Fig. 29. Submerged chlamydospores (†). Wort agar. × 350.

Fig. 30. Phialides with endoconidia. Sabouraud's dextrose agar. × 750.

Fig. 31. Hypha with warts. Sabouraud's dextrose agar. × 750.



PINKERTON — CONIDIAL FORMATION

WYNE, BOSTON.

Explanation of Plate

PLATE 5

Drawings made with the aid of a camera lucida.

Figs. 1–7. *Clonostachys spectabilis.*

Fig. 1. Terminal chlamydospores. Cherry agar. × 750.

Fig. 2. Intercalary chlamydospore. Malt extract broth. × 750.

Fig. 3. Submerged terminal cell (?). Malt extract broth. × 750.

Fig. 4. Botrytis type of spore. Sabouraud's dextrose agar. × 350.

Fig. 5. Same. Corn meal agar. × 350.

Fig. 6. Mycelium associated with Botrytis type of fructification. Czapek's agar. × 350.

Fig. 7. Spores. Corn meal agar. × 750.

Figs. 8–13. *Scopulariopsis brevicaulis.*

Fig. 8. Young conidium before putting down endogenous wall. Nutrient agar. × 1250.

Fig. 9. Mature conidium and phialide showing segmentation. Nutrient agar. × 1250.

Fig. 10. Conidium. Nutrient agar. × 1250.

Fig. 11. Branched conidiophore. Nutrient broth. × 1250.

Fig. 12. Phialide with conidia. Nutrient agar. × 1250.

Fig. 13. Conidia. Nutrient agar. × 1250.

Figs. 14–26. *Clonostachys Araucaria.*

Fig. 14. Chlamydospores. Corn meal agar. × 750.

Fig. 15. Mycelium with warts. Sabouraud's dextrose agar. × 750.

Fig. 16. Dimorphic conidiophore. × 350.

Fig. 17. Cephalosporium and phialide with endoconidia. × 1250.

Fig. 18. Phialide with twisted walls. Sabouraud's dextrose agar. × 600.

Fig. 19. Fructification like Corda's type. Sabouraud's dextrose agar. × 1250.

Fig. 20. Diagram showing a type of branching.

Fig. 21. Another type of branching.

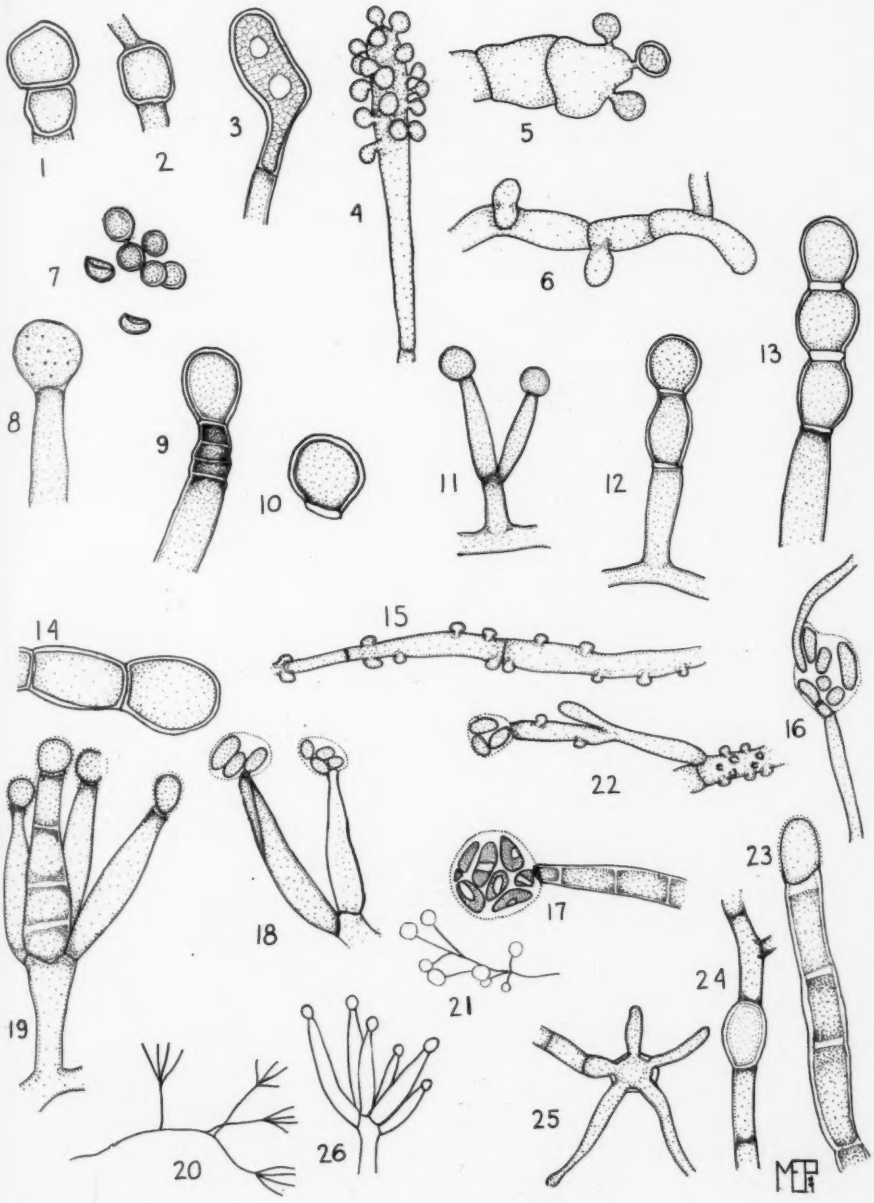
Fig. 22. Phialide with warts. Czapek's agar. × 350.

Fig. 23. Endoconidia. Sabouraud's dextrose agar. × 1250.

Fig. 24. Intercalary chlamydospore. Lactose broth. × 600.

Fig. 25. Germinating conidium. Lactose broth. × 600.

Fig. 26. Conidiophore with six concurrent branches. Wort agar. × 600.



PINKERTON — CONIDIAL FORMATION

AYNE, BOSTON.

EXPLANATION OF PLATE

PLATE 6

Figs. 1–14, giant colonies on Czapek's agar.

Fig. 1. *Cephalosporium niveolanosum*

Fig. 2. *Cephalosporium symbioticum*

Fig. 3. *Gliocladium deliquescens*

Fig. 4. *Gliocladium fimbriatum*

Fig. 5. *Gliocladium penicilloides*

Fig. 6. *Gliocladium Vermoeseni*

Fig. 7. *Allescheria Boydii*

Fig. 8. *Clonostachys Araucaria* var. *confusa*

Fig. 9. *Cephalosporium rubrobrunneum*

Fig. 10. *Clonostachys Araucaria* var. *rosea*

Fig. 11. *Clonostachys Araucaria*

Fig. 12. *Cephalosporium kiliense*

Fig. 13. *Cephalosporium Serrae*

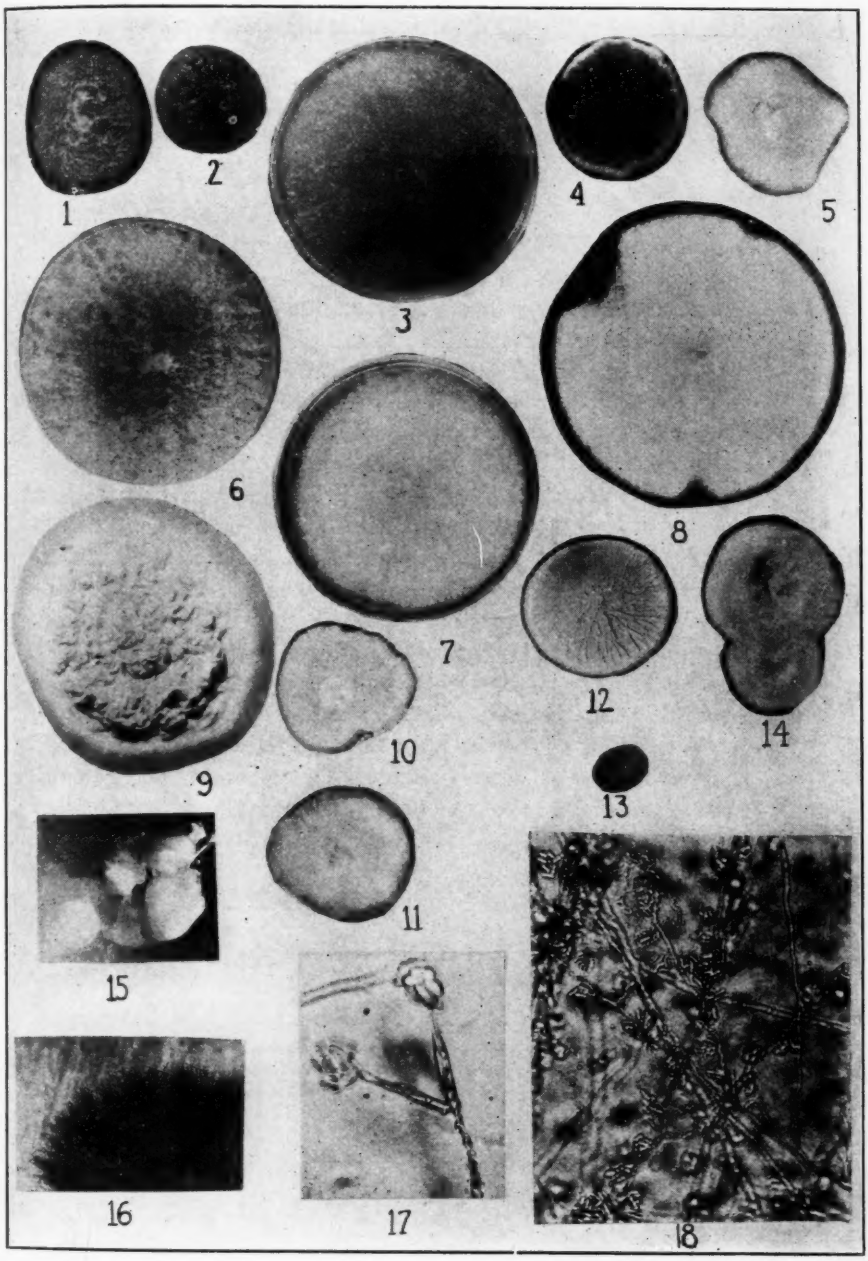
Fig. 14. *Cephalosporium Stuehmeri*

Figs. 15–18, photomicrographs.

Figs. 15–16. Granular incrustations of *Cephalosporium rubrobrunneum* upon blood serum.

Fig. 17. Branched conidiophore of *Clonostachys Araucaria* with warts and cephalosporia. × 1000.

Fig. 18. Agar micro-culture showing cephalosporia in *Cephalosporium symbioticum*. × 1000.



PINKERTON — CONIDIAL FORMATION

A STUDY OF THE GENUS HELICOGLOEA[1]

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A paper published by Patouillard in 1892 included amongst the heterobasidiomycetes the new genus *Helicogloea,* represented by a single species—*H. Lagerheimi* Pat. Later Patouillard seemed confused and uncertain as to its true identity and eventually, in a letter to Coker ('20), stated that *Helicogloea* was not distinct from *Platygloea.* In the literature subsequent references are based on Patouillard's work. No one seems to have examined the type. Just as this work was going to press under the name of *Saccoblastia,* Dr. Donald Rogers called attention to the type which is now in the Farlow herbarium. Patouillard's description fails to mention the "sac" or hypobasidium, easily the most conspicuous part of the fructification. From his illustrations one is forced to conclude that although Patouillard was aware of the structure he was unable to interpret it. There can be no doubt as to the real nature of the specimen. Not only is it a good genus but the type of what three years later Möller ('95) called *Saccoblastia.* Since Patouillard's formal description does not include a statement concerning the hypobasidia the genus stands in need of emendation. Accordingly, the recognized species of *Saccoblastia* must be transferred to the older genus, *Helicogloea.*

Helicogloea Pat., emend.

Fructification resupinate, indeterminate, ranging from a very thin film to a layer several mm. thick, mucous-gelatinous or

[1] An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

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floccose; hyphae with or without clamp connections, 2–6 μ wide, branched, sometimes anastomosing, attenuated at the septa; basidia heteromorphic, terminal or intercalary in origin; hypobasidia conspicuous, saccate lateral (with one exception) expansions of the basidial primordia sometimes variously constricted, 15–20–58 × 4.5–8–16 μ; epibasidia cylindrical outgrowths of the primordia or hypobasidia, expanding apically to the sporogenous portion, articulate-geniculate, 3–4-septate, each segment producing a sterigma and spore, or an accessory appendage eventually tipped by a sterigma and spore, the entire basidium 45–140 × 4–11 μ; spores hyaline, ovoid to ellipsoid, often flattened on one side, with a prominent blunt lateral apiculus, 7–19 × 4–13 μ; germination by repetition.

Helicogloea is a member of the Auriculariaceae, heterobasidiomycetes which bear basidiospores in linear sequence on transversely septate basidia. The genus, by reason of a curious sac-like appendage produced during basidial ontogeny, has always been noteworthy. Since the first clear morphological account (Möller, '95) several species have been described but little detailed information concerning the developmental cycle has accumulated. Gäumann and Dodge ('28) summarize the situation by saying: "A cytological study of the species is needed for the interpretation of this storage organ which is unknown in other groups of fungi."

In 1895 Möller *(l. c.)* described two species collected during a stay in Brazil. Both possessed the unique saccate hypobasidia. Möller completely delineated the morphology of the genus. Further detailed studies were not made. As apparently Patouillard's material was never questioned, this work of Möller's stood as the standard of the genus *Saccoblastia,* represented by the species *S. ovispora* Möll. and *S. sphaerospora* Möll.

The next record is that of Bresadola ('03), who added a species from Poland, *Helicogloea graminicola.* His description was accompanied by neither detailed studies nor illustrations. Likewise Bourdot and Galzin ('09, '28), who have enlarged the genus by several species and varieties, have contributed only taxonomic observations.

The first collection of the genus in the United States was made by Couch, 1920, in North Carolina. Coker ('20), puzzled by its peculiar characters, which were so suggestive of *Helicogloea Lagerheimi* yet differing in several respects, published a description of the fungus as *Saccoblastia ovispora* Möller var. *caroliniana*. In 1928, in the meantime having consulted Bresadola who supposedly had seen the type of the species in question, he decided to recognize it as an autonomous species, now *Helicogloea caroliniana*. The original description includes a careful morphological account with lucid illustrations.

Linder ('29) announced a new species based on material collected in Cuba, *Helicogloea intermedia*. This remarkable form was found to have two types of basidial development, the so-called "clavate" type in addition to the established saccate one. From his preparations Linder was able to augment considerably the cytological data. But even so they remained far from complete. The condition of his material which had been preserved in 70 per cent alcohol was hardly suitable for critical work.

Helicogloea Lagerheimi Pat. was collected in Iowa during the summer of 1932 by Rogers and later reported by him ('33). He made additional gatherings during the summer of 1934 and at both times killed and fixed samples of a number of collections. It is material from these sources upon which this study is based.

Technique

Several collections of fresh material of *Helicogloea Lagerheimi* were available for histological preparations. All of them were killed and fixed in Flemming's weaker fluid. To the fixing fluid for two lots was added 1 per cent maltose. For two others dehydration by a butyl series was employed, whereas the first mentioned were run through Chamberlain's ('32) full alcohol schedule to xylol and paraffin. Those of the maltose-xylol series proved the most valuable for critical stages. However, subsequent examination indicated that variation was due to the particular specimen as much as to fixation. Serial sections were cut at 15, 10, 8, and 5 μ. Various stains were tried but

Haidenhain's iron-alum haematoxylin with a counter stain of phloxine (a 1 per cent solution in 95 per cent alcohol) was used almost exclusively. A few slides were prepared with Flemming's triple stain to serve as a check for the presence of septa at certain points. In addition whole mounts were made of herbarium specimens whenever possible. After these were revived in a moist chamber they were treated with a weak solution of potassium hydroxide and stained with phloxine (1 per cent solution in water).

MORPHOLOGY OF H. LAGERHEIMI

The indeterminate fructification of *Helicogloea Lagerheimi* in a living condition, or when it has been revived in a moist-chamber, appears as a thin gelatinous or mucous coat, loosely attached to its substratum of decorticated and well-decayed wood of various sorts. It ranges in thickness from 100 μ to 1 mm. From prepared sections it is evident that the penetration within the substratum is slight. A few scattered hyphal threads are all that appear within the wood cells, and these are never deeper than a few μ.

Morphologically, there is no tissue differentiation into trama and hymenium, but the whole is simply a mass of hyphal and basidial elements loosely interwoven (fig. 19). This composite figure of the fructification as seen in section represents one in which the hyphae are practically parallel, a feature characteristic of lesser developed fructifications. The development of the fruiting body ranges from a thinly spread layer to thicker ones in which the basidial elements are restricted to the upper layer. In moderately developed specimens the fruiting layer may be as much as one-third the total, or in better developed ones as little as one-sixth, as shown by the comparative scales (fig. 20). The fruiting region is the actively growing part, bearing basidia singly at various levels. The individual hyphal cells are typically binucleate. Occasionally some cells are four-nucleate, but this merely indicates a recent nuclear division not yet followed by wall formation (fig. 14a, d). Usually the two processes are concurrent. The nuclei may lie

in close approximation or be widely separated within the same cell. As the cells become more mature the protoplasmic content is reduced, sometimes to only scattered remnants about the nuclei (fig. 14b, c). The mycelium is slightly irregular, characteristically attenuated at the septa, and its individual cells vary considerably in length. Branches arise as lateral outgrowths of a cell and receive daughter nuclei of the parent-cell dikaryon (fig. 15). There are no clamp connections, but the hyphae branch freely. Anastomoses also occur, although there is no evidence to support Linder's contention that such hyphal union carries a sexual stimulus to fructification.

The morphological development of the basidium may be followed in the diagrammatic series, figures 1 to 13 inclusive, and seen in summation in the composite illustration, figure 19. These figures, drawn to a common scale, show the entire cycle. The first indication of basidium production is the cutting off of a terminal hyphal cell, the primordial cell, easily distinguished by its more homogeneous and denser staining protoplasm (figs. 15, 1). It contains two nuclei, descendants of the dikaryon of the subterminal cell. There may be additional basidia produced by proliferation of the subterminal cell (fig. 16), just as shown by Möller in *H. Lagerheimi* and Coker in *H. caroliniana*. After an increase in thickness the primordium pushes out a small lateral protuberance just above the basal septum (fig. 2). This enlarges rapidly to become the "sac," or, as it will be designated henceforth, the hypobasidium, *sensu* Neuhoff ('24) (figs. 3, 4, 5, 6). Into this the two nuclei migrate and subsequently fuse.

Following a rather protracted resting period the fusion nucleus migrates from the hypobasidium, passing back through the primordial cell into the epibasidium, again *sensu* Neuhoff (fig. 7). The latter, an outgrowth of the primordium, may be apparent at an early stage. Furthermore, it may be distinctly elongated at the time the nuclei leave the hypobasidium or it may develop more slowly (figs. 7, 25). Between the primordium and the epibasidium there is commonly a conspicuous crimp or constriction, but never a wall (fig. 17). The epibasid-

ium is usually elongating as the nucleus moves along (fig. 8), and when it has attained its mature length the apical portion thickens and bends over (figs. 9, 10). Accompanying this is a complete evacuation and withering of the hypobasidium. Later the withering includes the primordial part, and eventually it extends to the lower portion of the epibasidium itself (figs. 11, 12). In mounts made with KOH these emptied portions may be quite swollen so that no collapse is apparent, but the regions are entirely drained of protoplasm. The nucleus now divides, the first septum coming in at once. In a short time the two daughter nuclei divide, and the typical segmented basidium is formed with four sterigmata and spores. These are so oriented commonly, through the curvature of the epibasidium, that the sporogenous portion lies procumbent on the surface of the fructification, thereby greatly favoring the chances of spore dispersal (figs. 12, 13). Bends are located below the spore-bearing portion or in it and simply adjust this region for favorable discharge (figs. 23, 63b). Excessively long thickened appendages in place of sterigmata occur frequently, but these eventually give rise to true sterigmata and spores (fig. 67b, c, d). Infrequently the basidia remain erect, in which case appendages take care of the spore orientation or if the sporogenous portion is unobstructed at the surface it need produce only sterigmata.

As would be expected, there is considerable variation in the morphology of the basidium and its parts. The lateral hypobasidium or "sac," the most conspicuous feature of the fructification, is typically oblong-ovoid, but often it is constricted once or more (fig. 18a, b), a condition culminating perhaps in the extremes illustrated by *f* and *g* of the same figure. It should be noted that the general orientation of the fructification is such that the hypobasidia are only rarely pendent as extant accounts say. The fructification is resupinate, commonly effused on the under side of logs, hence the general configuration is to be construed inversely to the usual interpretation (fig. 19). Usually the epibasidium originates from a terminal primordial cell as a vertical outgrowth from it, but occasionally it seems to have

been produced directly from the proximal end of the hypobasidium. The latter situation would then be equivalent to what apparently is the rule rather than the exception in *H. intermedia.* After examining Linder's material and his illustrations it is quite clear that there is a real difference in the origin of the basidial components of these two species. In *H. Lagerheimi* those hypobasidia which seemingly are not borne on terminal primordia with subsequent elongation of the epibasidia, but which appear to produce their epibasidia directly at their proximal end, are in reality borne on primordia which are short lateral branches of a proliferating complex. Hence their true origin is from a single terminal cell from which the hypobasidium arises in the ordinary fashion. This is clearly substantiated by the position of the walls (cf. figs. 21a, b; 75a, b). In *H. intermedia* one can easily trace the origin of the saccate type of hypobasidium from an intercalary binucleate cell which pushes out the hypobasidium, just as in *H. Lagerheimi,* and it in turn produces proximally the epibasidium (fig. 75b, c, d). There is, then, a fundamental difference between these two species in regard to the position and origin of the basidium and its components. In either species the type of development characteristic for one may occur as a rare exception in the other. Whether the primordium be terminal or intercalary, it is always distinguishable by its denser protoplasmic content. A study of *H. caroliniana, H. Lagerheimi,* and *H. graminicola* reveals that they are essentially identical at these stages. A third variation occurs in *H. pinicola.* Here the primordium is intercalary, but the epibasidium originates with a lateral outpushing of the primordium, usually more or less apical, rather than from the proximal germination of the hypobasidium (fig. 72b, c). No case of distal germination of the hypobasidium, such as Linder reported in his work, has been noticed in any of the material examined.

Quite early the budding epibasidium is visible at the apical end of the primordium. It is seldom distinguishable before the latter attains its mature diameter or previous to the formation of the hypobasidium, and it may not appear until much

later (cf. figs. 5, 22, 31b). Exceptionally, it is lateral in position. Its further development is either upright or variously bent, consequent upon its origin or the requirements for spore discharge (fig. 23). If there is a constriction between primordium and epibasidium it persists and is apparent in all stages, though there are numerous instances in which the epibasidium is little, if at all, distinct from the primordial portion (fig. 25b). The elongation of the epibasidium is various in relation to the accompanying nuclear behavior. It may follow one of three general patterns: I, The nucleus migrates from the hypobasidium as the epibasidium elongates (fig. 24); II, The elongation of the epibasidium distinctly precedes the nuclear migration (fig. 25a, b) which may be delayed until a stage as late as that shown in figure 10; III, Migration precedes elongation. In such event there is little if any protoplasmic evacuation of the hypobasidium until the epibasidium begins elongating (fig. 26a). The last scheme is infrequent except in less well developed fructifications. This results in a distinctly shorter basidium whose developmental sequence may be followed in figure 26b, c, d, and e. The other two types occur about equally, though type I probably represents the typical behavior. Type II seems more often associated with an extremely long unconstricted form of epibasidium and is undoubtedly a direct response to its location within the fructification. Types I and II intergrade. The type of behavior is consequent upon the primordium location. In general, if the primordium is near the surface there is no need for great elongation, but if it is well within the fructification the longer form necessarily results.

The crimp at the base of the epibasidium, though persistent, is never cut off by a wall. However, a second constriction is located characteristically just below the inflated sporogenous part. Such a joint has been noted by Coker in *H. caroliniana;* Wakefield and Pearson ('23) and Rogers ('34) show it in different degrees for *H. Lagerheimi.* According to Wakefield and Pearson there is no wall at this point; according to Rogers there is. Rogers' figures present a condition comparable to the one Coker noticed. In *H. Lagerheimi* three septa are found

more frequently than four in the sporogenous portion. Commonly, protoplasmic remnants simulate a basal wall, but the actual presence of a wall is exceptional (fig. 58). Ordinarily, the second constriction lies just behind the evacuation level of the fourth segment (figs. 12, 13, etc.); it may be accompanied by a bend. At times the constriction is very deep, and if in addition the fourth segment has a basal septum, the condition is identical with Rogers' illustration (fig. 27a, b). Whether the last segment does or does not have a basal wall, there is never any protoplasm left behind the segmented portion. In *H. caroliniana* the fourth septum is evident early in the development of the sporogenous portion (fig. 76b). Again the articulation receives varying emphasis so that one finds examples ranging from a definite joint, as Coker showed, to a simple bend.

Cytological Observations

Each hyphal cell characteristically contains two small nuclei whose position within the cell is various. The origin of the two-nucleate condition was not determined. As the cells grow older and elongate their protoplasmic content is much reduced until it may consist only of scant bits around the nuclei. The nuclei of the hyphae, however, remain small. Each nucleus is composed of a deeply staining central nucleolus embedded in the homogeneous nucleoplasm and is surrounded by a delicate membrane which is barely differentiated from the cytoplasm in which the whole lies. The apical cells destined to become the basidial primordia are limited to the more actively growing regions near the surface of the fructification. Their contents more nearly fill the cell and stain as an even, homogeneous mass. Consequently these primordia and the sub-adjacent cells are the most favorable places to locate mitosis. This is typically conjugate, both nuclei being entirely synchronized in behavior, though the smallness of the cells and of the nuclei at this stage makes detailed analysis practically impossible. At times one finds the spindles in the well-known side-by-side orientation or slightly oblique (fig. 28a). Curiously enough, in by far the

majority of cases the figures are crossed at varying angles (fig. 28b, c). Future orientation of the spindles in this way is implied clearly in the early stages shown by *a*, *b*, and *c* of figure 29. Such behavior is probably due to the small diameter of the cell which would prohibit a side-by-side arrangement. No additional details could be discerned except that the membrane has disappeared by the time of spindle formation, and sometimes the remnants of the nucleoli are visible in the nuclear cavity. Later they disappear (fig. 30). Following mitosis a wall is laid down between the two daughter dikaryons. This process may continue for several divisions but eventually the terminal cell constitutes the future primordium. The wall may not be formed immediately. In figure 31a, where it has just been laid down, the nuclei give evidence of a longer reorganization. Here, too, the cell contents are quite vacuolate, the result probably of rapid elongation and growth. The nuclei of the primordium are in a typical resting condition.

When first delimited the primordium is little distinguished in size from the hyphal cells. Before the production of the hypobasidium it increases in diameter and somewhat in length, though again it is difficult to evaluate this exactly in a form so variable in size and behavior. Examples in figure 32 represent an increase with reasonable surety, for they were approximately adjacent in the same section.

Beginning as a lateral outgrowth of the primordial cell just anterior to the basal septum, the hypobasidium rapidly assumes the form of an inflated sac-like appendage (figs. 31b, 33). At no time in its development is it without cytoplasm. As the hypobasidium elongates the nuclei in the primordium, which up to now have been more or less centrally located, enlarge slightly and prepare to move into the hypobasidium. They are now somewhat ellipsoidal. Sometimes the nuclei lie in such close association that they seem to be fusing, but careful focusing reveals that their individual membranes are intact (fig. 34a, b). One nucleus of the pair may precede the other into the hypobasidium by a considerable distance (fig. 35). In any event, the movement is always tandem, with the long axes

of the nuclei parallel to that of the primordium. There is no change in their shape as the nuclei pass through the narrow neck connecting hypobasidium and primordium. Following this migration the primordium is commonly more vacuolate (fig. 34b). Darker staining masses in the protoplasm are suggestive of a protoplasmic flow accompanying the nuclear passage (figs. 35, 37). Occasionally one nucleus of the pair may be larger, but this only signifies an earlier increase in its volume (fig. 38d, e). Once in the hypobasidium, the nuclei come together for fusion. Up to this time the hypobasidium has been growing but after karyogamy growth ceases. The two nuclei ordinarily do not penetrate below the mid-region of the hypobasidium, except in the case of the constricted hypobasidia where they always lie in the larger portion (fig. 18a, b, f, g). At the point of contact their membranes break down, allowing the components of the two nuclei to intermingle completely (fig. 38c). Usually until then their structure is no more differentiated than in the resting stage, although in figure 38d one nucleus is already forming a reticulum. A little later the fusion nucleus is dumb-bell-shaped but this constriction is transitory (fig. 39). The fusion nucleus is bounded by a continuous membrane; it contains two nucleoli, usually opposite each other, and a reticulum in varying degrees of advancement. The few variations of these earlier stages involve only minor deviations from the pattern. First, there is variability in the position of the nuclei in the hypobasidium. Figure 38e illustrates deeper penetration than usual. Another difference is that at times one nucleus is somewhat more advanced, as evidenced by its greater volume and earlier appearance of a reticulum (fig. 38e). In general these variations are rare, the migration and fusion processes being quite regular.

Within the fusion nucleus, now spherical in outline, the nucleoli draw together to unite (fig. 40a, b, c). The single resultant nucleolus clearly has an increased volume. By now the nucleus has very nearly attained its maximum size, with a diameter as much as five times that of the nuclei when in the primordium, almost equivalent to that of the hypobasidium.

Simultaneously the nuclear content is undergoing change. A sharp reticulum or network of threads develops throughout, highly suggestive of mitotic prophase. This increases in complexity until it reaches a stage illustrated in figure 41a. As the threads thicken, distinct, deeply staining beads representing aggregations of chromatic material are scattered throughout on them (fig. 41b). From this point on the nucleus is contracting and its entire contents become more concentrated and uniformly deeply staining. The single nucleolus gradually shifts from an eccentric to a centric position. This contraction is preliminary to a resting stage which will be followed by nuclear migration from the hypobasidium through the primordium to the epibasidium (fig. 42a, b). This contracting or post-fusion stage is the one most commonly seen; hence it probably involves a considerable span of time. With its contraction the nucleus becomes more and more homogeneous until it resumes the typical resting condition. When completely contracted the nucleus has a total volume not much in excess of the unfused nuclei at the time of migration but the nucleolar material quite evidently has been doubled in volume (see figure 71 for comparative nuclear and nucleolar ratios). The fusion nucleus ordinarily is in the center of the typical hypobasidium; in the constricted type it lies in the largest portion.

With the return to the resting condition the nucleus is ready to leave the hypobasidium. The primordial cell in the meantime has given rise to the epibasidium, more or less greatly developed. If the migrating nucleus is at all elongate the whole body moves with its long axis parallel to that of the hypobasidium, later to those of the primordium and epibasidium (fig. 43a, b, c). As in the earlier migration, there is no attenuation or change of form when the nucleus passes through the neck, for the diameter of the migrating nucleus never exceeds that of the neck. When the nucleus leaves the hypobasidium the protoplasm also withdraws, leaving the distal portion first, until the hypobasidium is completely emptied and withers. Evacuation eventually extends to the primordium and lower

portions of the epibasidium as their contents are withdrawn to augment the expanding sporogenous portion.

The epibasidial development at this time follows one of the plans already outlined. Of these the first two are by far the most common. In accord with these types, I and II, the first evidence for nuclear division is the enlargement of the nucleus followed by the appearance of threads in the heretofore homogeneous structure. This may begin at a level as early as that represented by figure 44b, but typically there are no signs of division until the nucleus comes to lie in the enlarged portion of the epibasidium, the actual sporogenous part (fig. 45a). By now all the protoplasm has withdrawn from the rest of the basidium into that region. There is seldom any further elongation or increase in diameter of the latter once division stages have been inaugurated. This first division of the secondary nucleus is taken to be meiotic. Once again the nucleus increases in volume, often nearly equalling the diameter of the tube, usually much elongated, in general conformable to the space in which it lies (figs. 46, 49). An increase in volume is the first diagnostic sign of the prophase and is the more marked because of the lighter staining properties of the nucleoplasm. This is followed by the gradual appearance of threads which soon become definitely organized. The membrane is intact about the threads and nucleolus. Chromatic beads appear on the threads but they are never as pronounced as those seen in the fusion nucleus (fig. 47). The threads thicken, become denser, and frequently are localized at a pole opposite to the nucleolus, even from the beginning (fig. 48a, b). The nucleolus is usually peripheral, rarely central. Stages represented in figure 48 may be interpreted as synaptic. Following this, the chromatic material is increasingly concentrated until it is massed more or less centrally in the nuclear cavity (fig. 48c). Slightly later, individual clumps are distinguishable in the mass and probably represent pairs or groups of chromosomes. The nucleolus, as yet distinct, from this point onwards decreases in size and finally disappears. No clear-cut evidence

was found for its direct expulsion into the cytoplasm. It usually is pushed to the edge of the nucleus quite early, and when the membrane breaks down lies free in the cavity or near by in the cytoplasm. Rarely is it distinct when the spindle is delimited; in one exceptional case a nucleolus was still quite sharp at anaphase. Commonly, what has been interpreted as a last remnant of it is seen in the cytoplasm (figs. 50b; 51a, b).

Development in accordance with type III must be considered a special case. There the prophase is distinct quite early in the development of the sporogenous portion, at times when the latter is just differentiated (fig. 45c) or even earlier. The nucleus here is less drawn out because it lies in a broader area. Aside from the earlier initiation point there is no further variation in procedure. The whole is simply a shortened basidium whose developmental processes as a consequence are likewise foreshortened.

A metaphase of the sort found in the higher plants has not been seen. As the chromatin mass separates into individual units, presumably chromosomes, a spindle is formed intranuclearly. It is always oriented parallel to the long axis, never oblique. Because of the extremely small figure, spindle fibers are not distinguishable, but the whole region appears simply as a darker-staining area. At the poles there are minute but definite dark-staining bodies, the centrosomes—more properly centrioles, for no further differentiation can be made. Their origin is unknown. Astral radiations do not occur. As the chromosomes pass into anaphase the nuclear membrane disappears, leaving the division figure in the nuclear cavity (fig. 50b, c, d, e).

At anaphase the chromosomes pass to the poles in a very irregular manner, for they may be so scattered in their distribution as to extend from equator to poles (fig. 50). It is during this scattering, if ever, that one can count the number of chromosomes present. The size of the figure and its chromosomes precludes any finality of count. As nearly as can be determined there are at least five pairs of chromosomes of different sizes.

Even though the membrane has broken down, the nuclear cavity, by virtue of its lighter staining properties, remains distinct for much longer, as figure 51c, a late anaphase, illustrates. There the spindle is stretched out and lengthened: the chromosomes are no longer discernible as individual units, for the majority are already massed together at the poles. There is no longer any trace of a nucleolus. The polar massing and the lengthening of the spindle continue until at telophase a condition represented by figure 52 is characteristic. The connective fibers or strands between the two polar masses are conspicuous. Similar persistent strands have been noted in the fungi by numerous authors. Tischler ('21–'22) designates them "karyodesmotische," Bensaude ('18), "pont cinoplasmique," and Colley ('18), "suspension fibers." That their occurrence is far from uncommon is evident from the frequency of reports covering a wide distribution of forms (cf. Blackman, '04, Colley, '18, Lindfors, '24, and Pady, '34, on rusts; Maire, '08, on divers basidiomycetes; Neuhoff, '24, on the secondary nuclei of Auriculariaceae and Tremellaceae). These connections are still distinct when lighter areas around the now almost structureless chromatic masses give clear indication of the reorganizing daughter nuclei (fig. 52). Gradually a delicate membrane surrounds them. Apparently the chromatic masses prominent at telophase become the nucleoli of the daughter nuclei, a situation commented upon by Sass ('29) in his studies of Agaricaceae.

The two nuclei are usually synchronized in their behavior but now and then one matures in advance of the other (fig. 56a, b, c). A wall separating the two nuclei either is laid down before reorganization or much later. It is distinguishable at first only as a very delicate deposition, barely differentiated from the surrounding protoplasm; occasionally a clearer zone seems to mark its future position. Delay of the wall formation until the nuclei are undergoing the second division is the exception (fig. 56b). As nearly as could be determined the wall is formed by uniform deposition. Frequently a heavier aggrega-

tion is made out mid-way on the spindle strands. This may represent the initiation of the new wall (figs. 52d, 56b).

Daughter nuclei from the first division never reach a particularly high degree of reorganization, for the second division follows almost immediately. That this does ensue rapidly is inferred by the fact that early-division stages of it were never seen. Spindle orientation is longitudinal, with only an occasional and minor deviation to the oblique. Anaphases with characteristic spindles and darker-staining bodies, the chromosomes, are common (fig. 55). In figure 56a the nucleolus of one dividing nucleus has been cast aside and is disintegrating. The subsequent behavior resembles that of the first-division figures and leads to the formation of four nuclei. Moreover, the spindles are notably smaller than those of the previous division and therefore the chromosome number could not be determined with any degree of satisfaction. An exactly parallel situation has recently been recorded by Pady ('35) for the division of the fusion nucleus in the promycelium of *Hyalopsora*. Ordinarily the two nuclei progress at equal rates, but when they are not synchronous the basal segment may have a nuclear phase as advanced as late telophase while the upper is still in anaphase, or vice versa (fig. 56a). Again a spindle may not have formed in one, although the second is already in anaphase. When all four nuclei are reorganized there results the typical segmented spore-bearing part (fig. 57). In that figure the mid-wall, the first one formed, is clearly older and better developed; the other two have just been laid down. As already noted, a fourth septum is exceptional, but if present it appears later at the base of the fourth segment. Figure 58a shows a true septum but figure 58b illustrates how protoplasmic remnants may simulate it.

The four daughter nuclei often display an inequality of size, that of the basal segment being subject to the greatest variation, for it is often noticeably smaller than the others. These nuclei are all in a resting condition. Though their total volume now is slightly greater than in resting nuclei elsewhere, the nucleolus is no longer enlarged. Here, if ever, two nucleoli

may appear, though apparently the condition is evanescent (fig. 59). This is in accordance with the already ascertained fact that nuclei may show variability in this respect, especially after several successive divisions, such as is the case here (Sharp, '34). That there is great variability in the nuclear and nucleolar volumes all through the cycle is evident (fig. 71).

Sterigmata promptly appear as small outgrowths from each segment (fig. 60). They are not formed synchronously nor in any regular order of succession. In most instances the fourth segment is the last to produce its sterigma, but it may be the first segment in which production is longest delayed. Initiation commonly starts in the second unit (fig. 61). There is no correlation between the position of the hypobasidium and the side of the sporogenous portion which produces the spores, but the spores are borne on the convex side. Sometimes the end segment bears its sterigma apically (fig. 63a); infrequently sterigmata may be borne on opposing sides (fig. 63d). At this time the nuclei are usually to be found directly opposite the budding sterigmata, more often peripheral than central. Gradually the sterigmata lengthen, and then small vesicles, the future spores, appear at their apices (fig. 62).

Sometimes the vesicle is preceded by a dark-staining cap or plug whose nature was not determined (fig. 64a, b). Colson ('35) finds a similar condition during early sterigmatic production in *Psalliota campestris,* where a small quantity of stainable material appears in the narrowest region. She considers it merely a mechanical retention of stain. Such would also seem to be the case here. Only the initial spore stages are hyaline, and the expanding spore is henceforth never without a definite protoplasmic content. Protoplasmic migration from the basidium segments begins early and precedes nuclear migration, which does not take place until the spore is practically mature. The latter induces striking changes in the nucleus. While the spore is forming the nucleus becomes decidedly more stainable and completely altered in shape (fig. 65a–d). Finally it becomes very dense, staining homogeneously black, and by then is quite elongate and more or less irregular in outline. It

moves into the sterigma as an undifferentiated compact mass (fig. 66a, b). Often it is long and slender during its progress into the spore (fig. 66c, d). This is not an uncommon phenomenon in the basidiomycetes. As the nuclear mass penetrates the spore the latter may show vacuolation. By now the protoplasm is well withdrawn from the segment, the last remnants passing in with, or just after, the nucleus. No protoplasmic lining ever remains in the cells as Buller ('22) noted in the Auricularieae. Once within the spore the nucleus undergoes corresponding changes in reverse order until the true resting nuclear state is again attained (fig. 66e, f, g, h, i). The volume is slightly under that of the antecedent resting condition.

In discussing the general morphology, the formation of exceedingly long thickened appendages in place of sterigmata was touched upon. At first it was thought that these might be indicative of direct germination as the nucleus early moved into them without undergoing change. But later it was found that they result directly from the position of the basidium and are merely a means of elevating the spores to the surface. Their consistent occurrence in regions of greater compactness in the fructification bear this out. Sometimes the lower segments of a basidium have these appendages, whereas the upper one produces an ordinary sterigma (fig. 63a). Eventually the appendages produce true sterigmata and spores, terminally. This procedure is well known in other heterobasidiomycetes; for example, in *Auricularia* the long appendages push through the gelatinous matrix to bring the sterigmata and spores to the surface. In the rusts normally (Gäumann and Dodge, *l. c.*) as well as under unfavorable conditions, the basidiospores are produced only when brought to the surface. The nuclei then move into the spores, having undergone the changes described. These nuclear changes may begin early, the initiation point being correlated with the length of the appendage (fig. 67e, f). The nuclear behavior is in complete agreement with Neuhoff's criterion for a sterigma. Linder speaks of variation in sterigmatic length in *H. intermedia* (see his pl. 41, figs. 13–15), a situation which is probably tantamount to the one just described.

Germination, which may take place at once, begins with the emission of a short germ tube and a sterigma upon which a secondary spore exactly like the first is borne (fig. 68b, c, d). This is the so-called "germination by repetition," long a recognized character of the heterobasidiomycetes (Patouillard, '87). Not only is the morphology of the sterigma the same but the nucleus, in order to pass into the secondary spore, is altered in exactly the same fashion as characterizes the passage from basidial segment to spore (fig. 69). Nuclear division within the spore has never been seen. At times spores begin germinating while still attached to the sterigmata (fig. 68b). No data pertaining to the actual spore discharge were obtained.

Attempts to culture *H. Lagerheimi* have been unsuccessful so far. Unfortunately no fresh material was available, and herbarium specimens of the 1934 collections had to be relied upon. Spores from that source sown in hanging-drop cultures of sterile distilled water gave a goodly percentage of germination within from 24 to 48 hours, but transfers weré never successful. From observation of spore behavior in the hanging-drop cultures it is readily seen that the usual mode of germination is by repetition. Möller illustrates septate spores, each segment of which is capable of producing a secondary spore. This has not been seen. Although in Patouillard's type material a spore with two germ tubes was found, septation between was indistinct. The spores do occasionally segment in germination, but their subsequent behavior was not determined. Small sub-globose conidia appear in older cultures. Their mode of origin was not seen. Frequently they are massed, just as Möller reported.

Before the appearance of the germ tubes the spores are quite hyaline, in most cases with a single, rather conspicuous oil globule. As germination processes are initiated this divides into two to many with corresponding reduction in size. When the germ tube arises the contents of the spores are distinctly granular with numerous oil bodies. The tubes themselves are mostly lateral, but may be terminal, in position. At their apices a small bubble-like protuberance is the first sign of the

secondary spore. This remains quite hyaline until maturity when the content of the original spore is discharged into it. In some cultures within a few days delicate mycelial outgrowths were evident (fig. 70).

TAXONOMIC CONSIDERATIONS

All the species of *Helicogloea* are entirely saprobic. They are found on well-decayed woods of various kinds, including pine, alder, birch, beech, oak, aspen, poplar, and willow, rarely on humus or soil. One species occurs on grasses from whence it takes its name, *H. graminicola*. Collections have been made of some species practically throughout the year; others seem to have more seasonal limitations. Any is more likely to be found in damp rather than dry weather. Geographically, the distribution is scattered. *H. Lagerheimi* is the most widespread. Besides the type locality, Ecuador, it has been reported from France, England, and the United States. The only other North American species are *H. caroliniana*, as yet found only in the type locality, North Carolina, and *H. pinicola* recently reported from Canada.

As pointed out earlier, the chief considerations of the genus have been taxonomic. The most comprehensive treatment is that of Bourdot and Galzin. They characterize the genus as "floccose or gelatinous-mucous, spreading; the probasidium in the form of a sac, laterally pendent and giving rise to a basidium with three transverse walls; sterigmata lateral (one terminal); spores hyaline, smooth." By reason of the floccose or gelatinous nature of the parent genus, these authors have erected the subgenus *Saccogloea* to care for the mucose-gelatinous forms. Furthermore, they comment: "The French species of *Saccoblastia* do not always show the generic characters clearly; the contents of certain probasidia appear often to be a reserve utilized for the formation of the basidium, but it seems in many cases that it is the probasidium itself which becomes erect and is directly transformed into a basidium. At least, the section shows often all the intermediate stages between the probasidium as a pendent sac and the curved or erect

basidium." In view of such indefiniteness all obtainable species were studied. A brief description is given of each, chiefly for its morphology.

HELICOGLOEA **pinicola** (Bourd. & Galz.), comb. nov.
Pl. 13, figs. 72a–l, 73a–c.
Saccoblastia pinicola Bourdot & Galzin, Soc. Myc. Fr. Bull. **25**: 16. 1909.

The type has not been seen, but two collections of Galzin's, and Bisby's Canadian gathering were examined. They are all truly floccose. The scattered receptacles of Galzin's collections are Cartridge-Buff.[2] A few patches here and there, gelatinous in consistency, are a deeper color—Olive-Buff to Deep Olive-Buff. The Canadian material shows no gelatinous areas.

This species is clearly a member of the floccose section. Bourdot's and Galzin's description answers with some exceptions. The hypobasidium was found to be consistently larger than the range given by these authors. They say it is 40–45 × 8–12 μ; measures made here extend the range to 19–56 × 7–14 μ, the majority in Galzin's material falling around 50 μ in length. The morphological plan is even less in agreement. Bourdot and Galzin refer to variation in the "basidia" but not to any in the hypobasidia. In the material studied the latter is extremely noticeable. Irregularities most frequently take the form of forked or branching hypobasidia (fig. 72a, i, j). Forked basidia mentioned and figured by Bourdot and Galzin have not been found, but figure 72i, for example, strongly suggests that these authors may have mistaken forked hypobasidia for forked basidia. The most striking feature is the mode of origin of the basidium parts. Occasionally the primordium is a terminal cell of varying length, as in *H. Lagerheimi,* but usually it is intercalary. The hypobasidium arises in the usual way as a lateral outgrowth but at maturity presents a great variety of forms—from straight to constricted to forked (fig. 72). The size is usually great in comparison with those of other species. The epibasidium is produced as a lateral

[2] Capitalized color names are from Ridgway, R., Color Standards and Color Nomenclature. 1912.

out-pushing of the primordium at or near its apex. Hence one commonly finds the basidia appearing in chains in contrast to the type of proliferation common in *H. Lagerheimi* (fig. 73e). At maturity the spore-bearing region of the epibasidium is three- or four-septate, and the portion posterior to it withers. Apical germination of the terminal segment is common (fig. 72e, k).

Bourdot and Galzin say this species is "very close to *H. graminicola*, of which it is perhaps only a variety. It differs in habit and the basidia and spores are notably larger." There seems to be little basis for the assumption of varietal status. True, both species have conspicuous clamp connections and both are supposedly floccose, but the epibasidial character and origin in the two are entirely different, nor does *H. graminicola* ever show the hypobasidial irregularities so marked in *H. pinicola*.

A collection from Litschauer of this species on *Alnus viridis* undoubtedly belongs to the form *alniviridis*, although it was approved by Bourdot before he segregated the form. It shows no deviation from described characters except that there was no paucity of hypobasidia such as Bourdot reported. Morphologically it is identical with the species (fig. 73 a–e). The validity of forms founded on substratal attachment is questionable for pure saprobes, even more so in this case since the advent of Bisby's specimen which was found on *Populus*. It, too, showed no specific differences.

FRANCE: Causse Noir, April 25, 1910, *Galzin, 20555* (in Herb. Univ. N. Car. ex Herb. Bourdot); Nov. 19, 1916, *Galzin, 2104* (in Farlow Herb. ex Herb. Burt). AUSTRIA: Tirol, Aug. 15, 1924, *Litschauer* (in Herb. Univ. Iowa). CANADA: Manitoba, Victoria Beach, Aug. 23, 1935, *Bisby, F5695*.

HELICOGLOEA **graminicola** (Bres.), comb. nov.

Pl. 13, figs. 74a–d.

Saccoblastia graminicola Bresadola, Ann. Myc. 1: 112. 1903.

After this is moistened it appears as a thin gelatinous coating on a grass stem, in color Vinaceous-Buff. There is nothing in its structure or consistency to justify Bresadola's

adjective "tomentose." From phloxine-stained mounts one can readily distinguish its salient characters. Micro-dimensions show close correspondence to those given by Bresadola. The primordium is terminal and it gives rise to the epibasidium. A conspicuously elongate connection between primordium and sporogenous portion is not found. The hypobasidia are large, coarser in appearance than those of *H. Lagerheimi*, more constant in size than those of *H. pinicola*, and are never forked. All the protoplasm withdraws from the hypobasidium but not necessarily from the primordium, which may still show a definite protoplasmic content after the segmentation of the spore-bearing region (fig. 74c). Clamp connections are prominent on the hyphae, sometimes appearing on the basal septum of the primordium which just beyond on the opposite side is producing the hypobasidium (fig. 74a).

Eichler (in Farlow Herb. ex Herb. Bresadola, ex Herb. Patouillard).

HELICOGLOEA **intermedia** (Linder), comb. nov.

Pl. 13, figs. 75a–h.

Saccoblastia intermedia Linder, Ann. Mo. Bot. Gard. **16**: 488. 1929.

A slide of the type material prepared by Linder was studied for the morphology of the species. From this it was clear that the origin of the saccate hypobasidium is intercalary, not terminal, or only exceptionally so. Following nuclear fusion within the hypobasidium, the latter germinates proximally to the epibasidium. After great elongation four sterigmata and spores are formed on the thickened end. Linder uses the terms "probasidium, promycelium, and basidium" for these parts. However, there is no reason, in spite of morphological variation, why Neuhoff's terminology is not applicable. The clavate type simply represents direct development from a terminal primordium. In this event, when a lateral sac is not produced, fusion takes place in the clavate portion itself; hence it is to be interpreted as the hypobasidium and its subsequent outgrowth as the epibasidium. Figure 75 is included merely for comparison with the other species.

Linder speaks of the saccate hypobasidium exhibiting distal germination at times. If his figures (pl. 40, fig. 4; pl. 41, fig. 9) represent the best evidence for that phenomenon, its occurrence is questionable. Succeeding stages would be convincing but none was found. However, the hypobasidia are frequently constricted, once or more, and it is possible that this so-called distal germination is only an extreme constriction. Distal germination was never seen in *H. Lagerheimi*. Hypobasidia of the form illustrated in figure 75e, *H. intermedia*, might be interpreted as unusually constricted, especially when compared with figure 18f and g of *H. Lagerheimi* which is so construed. Furthermore, if the orientation of the two fructifications is the same, then distal germination could only occur for those rare cases where the hypobasidia are lateral or pendent.

CUBA: Soledad, Sept., 1924, *Linder*, TYPE (in Herb. Mo. Bot. Gard.).

HELICOGLOEA **caroliniana** (Coker), comb. nov.
Pl. 13, figs. 76a–c.

Saccoblastia caroliniana Coker, Jour. Elisha Mitchell Sci. Soc. **43**: 233. 1928; *S. ovispora* Möller var. *caroliniana* Coker, Jour. Elisha Mitchell Sci. Soc. **35**: 121. 1920.

Coker's descriptions are so adequate that little need be said here. The fructification is a particularly well-developed gelatinous one. In its morphological behavior it resembles *H. Lagerheimi* very closely. A series of diagrams is included for comparison. Here, as in the other species of the genus, long appendages may precede the formation of sterigmata and spores (fig. 76d).

NORTH CAROLINA: Chapel Hill, April 15, 1920, *Couch, 4256* (in Herb. Univ. N. Car.); Chapel Hill, July 31, 1920, *Couch, 4601* (in Herb. Univ. N. Car.).

HELICOGLOEA LAGERHEIMI Patouillard, Soc. Myc. Fr. Bull. **8**: 121. 1892. Pls. 7–12; pl. 13, figs. 77–78; pl. 14.

Saccoblastia ovispora Möller, Protobasidiomyceten, Bot. Mitt. **8**: 16. 1895.

Saccoblastia sebacea Bourdot & Galzin, Soc. Myc. Fr. Bull. **25**: 15. 1909.

Saccoblastia sebacea var. *vulgaris* Bourdot & Galzin, Hym. de France, p. 5. 1928.

Saccoblastia sebacea var. *pruinosa* Bourdot & Galzin, Hym. de France, p. 5, 1928.

A study of the type material resulted in the above changes in synonymy. Figures 79–81 show the development of the basidium as seen in Patouillard's material, from the initiation of the primordium to the mature basidium and spores. Though micro-dimensions are consistently larger than those from the majority of collections, they do not exceed the uppermost figures obtained. There is no uncertainty about the identity of this material with Möller's or Bourdot's and Galzin's. Therefore the earliest name takes precedence. On the basis of basidium size *H. caroliniana* is perhaps the nearest species. But the two are so totally different in their habit they could never be confused. In particular, the hypobasidia of the type collection are sometimes constricted and frequently are set farther forward in relation to the basal wall of the primordium (fig. 79d) than usual. No case of intercalary origin was noted in this material.

Saccoblastia sebacea B. & G., an acknowledged gelatinous form, has been separated from *S. ovispora* Möller largely because the latter was supposedly floccose or hypochnoid. A study of all available specimens and descriptions has been made in an effort to straighten out the situation.

Of *Helicogloea Lagerheimi* (*Saccoblastia ovispora* Möll.), Möller wrote: "Sie bildete einen dünnen, fast durchsichtigen lockeren weissen Ueberzug, der in ganz unregelmässiger Umgrenzung mehrere Centimeter in jeder Richtung sich ausdehnte. Bei sehr feuchtem Wetter sieht dieser Ueberzug fast schleimig aus, da das Gewirr der Fäden Wasser zwischen sich festhält, bei trocknerem Wetter dagegen bemerkt man nur einen lockeren Hyphenfilz, der bei vollständigem Trocknen zur Unsichtbarkeit zusammenfällt." (l. c.). Ferry ('96) the following year translated Möller's work into French, in addition utilizing Möller's plates. The structure of the fructification is described as irregular patches of white loosely interwoven hyphae. Lindau ('97) introduced the adjective "wer-

gartig" in his characterization. As yet only Möller's species were known. Patouillard ('00) delineated the genus *Saccoblastia* as floccose, soft, and said of the tribe Septobasidiés to which he assigned it: "Floconneux ou crustacé, non gélatineux." Chronologically Bresadola's ('03) report is next, which described *H. graminicola* as "tomentose." Clements ('09) placed the genus (as *Saccoblastia*) in that group of Auriculariae having a byssoid pileus. Clements and Shear ('31) adhere to this characterization. Saccardo ('82) qualified the structure by saying, "Est quasi *Hypochnus* sporis pleurogenis." Killerman ('28) repeated Lindau's diagnosis for two species, having replaced *H. sphaerospora* with *H. graminicola*. The character of floccose as opposed to gelatinous is the distinguishing one for two sub-genera in Bourdot and Galzin.

Thus there had gradually crept into the literature the conception of floccose or hypochnoid fructifications in *Helicogloea*. *H. pinicola* is truly floccose. The interpretation of *H. Lagerheimi* as floccose probably has been considerably strengthened by Bresadola's comment to Coker in reference to *H. caroliniana* which was first described as a variety of *H. Lagerheimi*. Coker says *(l. c.)*: "I later sent some to Bresadola who has described a sp. of *Saccoblastia* and seems to have seen *S. ovispora*. He writes as follows: 'For me it is a species quite distinct from *S. ovispora*. The genus *Saccoblastia* is not gelatinous; your species is gelatinous like *Platygloea;* . . .'"

An exactly parallel situation has arisen in the case of *Stypella minor* Möller and *Tremella gangliformis* Linder. As Martin ('34) points out, the error is not in Möller's original description but in subsequent misplaced emphasis and consequent distortion. Since the type of *Helicogloea Lagerheimi* is mucous-gelatinous, all necessity of maintaining these species separately is removed. Unfortunately, Möller's type specimen has not been located; Rick's material, agreeing well with Möller's descriptions and coming from an adjacent province, gives strong likelihood of being the same fungus. The type of Bourdot's and Galzin's species likewise has not been seen, but all the collections so labelled which have been at

hand were decidedly not floccose. When any of these specimens is moistened it is mucous-gelatinous, but as it dries one can trace just such a sequence as Möller described.

Throughout the genus there seems to be not only inconsistency of interpretation but a real variation in the nature of the fructification itself. Wakefield and Pearson ('23) note of *H. Lagerheimi* that a series of specimens shows the "mucous-gelatinous" texture only in later stages. "When at its best the fungus has somewhat the consistency of *Corticium confluens*, and is beautifully pruinose with the projecting basidia and spores." Rick (personal correspondence, 1934) says: "The *Saccoblastias* are surely hypochnoid without true hymenium, but when well developed they are gelatinous, like *Exidiopsis*, and dry a little glancing." To Rick obviously hypochnoid connotes structure rather than consistency. On that basis all the species are "hypochnoid." It is significant that he considers the genus gelatinous, even though earlier he had been in accord with conventional treatments when in a monograph of protobasidiomycetes ('33) he referred the genus to the floccose Auriculariales. As already remarked, *H. pinicola*, which is distinctly tomentose for the most part, has some scattered gelatinous patches.

Möller's statements may have left latitude for differing interpretations, but his figures are clear-cut and easily show the close resemblance to *H. Lagerheimi*. A table of measurements for the specimens under discussion shows there is no reason to maintain any as distinct species because of variation in size. Only in the event that Möller's type proved to be distinctly floccose would it be reasonable to retain that species.

Two collections from Brazil were studied, both Rick's. The first of these was quite inconspicuous until moistened, when it became visible as thin gelatinous film, faintly pustular, of a Buffy-Brown color; no part of it could be interpreted as floccose. Unfortunately, the specimen was so contaminated with hyphomycetes that mounts were far from satisfactory, the more so as it did not seem to be in a particularly advanced fruiting condition. However, all evidences indicated a struc-

tural development much like the type of *H. Lagerheimi.* The mycelium is typical; oblong-ovoid hypobasidia—straight or constricted—are borne laterally on terminal primordia (fig. 78a). There is rarely deviation from the typical epibasidial production (fig. 78e).

The second of the collections may easily be a portion of the first, to judge from appearances. Certainly when examined before moistening it is not at all floccose. If discernible at all when dry it is only as a dried mucoid layer spread thinly on the bark substratum. As it revives in a moist-chamber it quickly swells into a mucous-gelatinous layer, exactly like that of the first collection. This material offered a greater range of basidia, though mature ones were scarce. Very infrequently basidia of intercalary origin were found. The hypobasidia are often constricted. Figure 78 a–g outlines the structural plan for both collections.

Examination of the other herbarium specimens suggested emphasis of the following points. Usually the primordium of the basidium is of terminal origin, rarely it is intercalary. The hypobasidia are straight or constricted, but in a few collections irregular ones approaching the *H. pinicola* type occur. Microdimensions show a greater range than those given by Bourdot and Galzin, but they are fairly consistent for the particular collection. All the fructifications are decidedly gelatinous with a color range from Ochraceous-Buff to Fuscous, most commonly Grayish-Olive.

Bourdot and Galzin have included several varieties. Opportunity to examine a large number of collections of the species and the varieties *pruinosa* and *vulgaris* from Bourdot's herbarium showed that the varietal segregates are untenable on both habital and structural bases. According to Bourdot and Galzin, the variety *vulgaris* differs only in that it is "thinly spread in large gelatinous patches." When moistened it so appears; its color is pallid Cinnamon-Buff or Grayish-Olive. There is scarcely anything distinctive about this; certainly in structure it is identical with the parent species as shown by figure 77.

The variety *pruinosa* is segregated by these same authors on the bases of micro-dimensions and growth form. The measures of the hypobasidia in the material examined slightly exceed those cited by Bourdot and Galzin. Spore size extends from 8–11.5 × 5–6.5 μ. Though the spore size of the parent species ranges from 8–18 × 4–9 μ, the majority fall in a middle grouping around 9–11 × 5–7 μ. It would be impossible to make a separation on spore size. The micro-dimensions do not distinguish the variety from the parent species; morphologically there is no difference between the two, and the color range is also closely parallel. No illustrations are included.

From the table following it is clear that there is more reason to set aside separate varieties than to maintain the former species because of differences in measurements. Such a criterion is hardly reliable in a group as variable as the heterobasidiomycetes.

HELICOGLOEA LAGERHEIMI PAT.

	Hyphae	Hypobasidium "sac"	Epibasidium	Spores
Type	2–6 μ	18–38 × 5.5–10 μ	69–100 × 5–7.5 μ	13–15–18 × 6.5–8 μ
Möller's measures	2–6 μ	30 × 8 μ	100 μ	13 × 7–9 μ
Rick's colls.	2–6 μ	18–33 × 6–7.5 μ	65 × 4.5 μ	15–18 × 6–7.5 μ
B.'s & G.'s measures	2–6 μ	18–30 × 6–9 μ	45–75 × 4–6.9 μ	8–10–15 × 5–6–8 μ
Other specimens	2–6 μ	15–39 × 5–13 μ	58–105 × 5–9 μ	8–12–17 × 4–9 μ
var. *vulgaris*	2–6 μ	18–28 × 5–8 μ	50–60 × 5–6 μ	9–11 × 6–7 μ
var. *pruinosa*	2–6 μ	16–32 × 5–9.5 μ	50–60 × 5–6 μ	8–11.5 × 5–6.5 μ
Roger's measures	3–4.5–6 μ	16–32 × 7–10 μ	75–100 × 4.5–7.5 μ	11–14.5 × 5–7.5 μ
Total range	2–6 μ	15–39 × 5–13 μ	45–105 × 4–9 μ	8–18 × 4–9 μ

ECUADOR: Chorrera de Agoyan near Baños, Jan. 1892, *Lagerheim*, TYPE (in Farlow Herb. ex Herb. Patouillard).

BRAZIL: Rio Grande do Sul, São Leopoldo, 1931, *Rick* (in Farlow Herb.); 1931, *Rick* (in Herb. Mo. Bot. Gard.).

UNITED STATES: Iowa, Iowa City, Oct. 5, 1932, *Rogers, 242*; July 8, 1934, *Rogers*,

305, 306; July 9, 1934, *Rogers, 274;* Missouri, Springfield, Oct. 2, 1933, *Looney* (all in Herb. Univ. Iowa).

FRANCE: Allier, *Bourdot, 5750* (in Farlow Herb. ex Herb. Patouillard); *Bourdot, 5750, 5882, 39135* (in Herb. Paris ex Herb. Bourdot); Aveyron, *Galzin, 3895, 3951, 4334, 5749, 7856, 7961, 10085, 11982, 13816, 13817, 14003, 14247, 14270, 14368, 14411, 14606, 14764, 14790, 14800, 14825, 14828, 16605, 16632, 16638, 16641, 16646, 16647, 16704, 16933, 17210, 17454, 18828, 18899, 19067, 20134, 21132, 23717, 23718, 23719* (in Herb. Paris, 23305, 6017, 6508, 7612, ..., 23288, 23289, 23240, 12151, 12153, 12349, 12351, 12350, 23291, 23308, 23333, 23293, 12348, 12411, 23294, 23295, 23302, 23297, 23296, 23300, 23303, 23301, 23304, 23298, 23335, 14915, 15419, 15479, 15991, 18561, ..., 24120, 24121, 24122); St. Guirol, May 6, 1910, *Galzin, 5815* (in Herb. Paris, 23287 ex Herb. Bourdot); Tarn, July 23, 1909, *Galzin, 4316* (in Herb. Paris, 23306 ex Herb. Bourdot); April 10, 1916, *Galzin, 19561* (in Herb. Paris, 2331 ex Herb. Bourdot); St. Priest-en-Murat, Nov. 1914, *Bourdot, 42262* (in Herb. Univ. N. Car. ex Herb. Bourdot); Orne, Oct. 1925, *E. Gilbert, 1313* (in Herb. Paris, 39816 ex Herb. Bourdot).

ENGLAND: London, Feb. 1922, *Pearson* (in Herb. Paris ex Herb. Bourdot).

DISCUSSION

Mitotic processes of *Helicogloea* give no indications of abnormalities, although a few stages encountered need some exposition. The unusual spindle disposition in nuclear division is by no means restricted to this form. Kniep ('13) noted a similar irregularity in *Hypochnus terrestris*. His figures include a definitely crossed type and one slightly angled, both from a germ tube; and the usual conjugate type from the mycelium. This gradation is comparable to that found in the terminal hyphal cells of *Helicogloea*. Tischler *(l. c.)* adopted Kniep's figures; neither author offers an explanation. Neuhoff ('24), throughout his studies on Auriculariaceae and Tremellaceae, saw mitosis only once—in *Achroomyces Tiliae*. Quite obviously, his illustration denotes that the spindles were crossed, but of this he makes no mention. However, he regards as abnormal crossed spindles in the hypobasidial division in *Exidia pithya*. This same phenomenon has been demonstrated frequently in the divisions of the fusion nucleus and in one instance of nuclear division in the spore (cf. Rogers, '32; Lander, '35; Colson, '35). That the clamp connection is a device to permit the side-by-side orientation of the spindles for the conjugate division of the dikaryon and a means to bring non-sister nuclei together, is widely accepted. Since there are

no clamp connections in this species, it is perhaps reasonable to assume that the crossing of the spindles is imposed by the narrow limits of the cell diameter.

Sass ('29) has commented upon the fact that apparently the chromatin masses at telophase become the nucleoli of the daughter nuclei. This is quite evidently so in the meiotic divisions of *Helicogloea* where a complete sequence can be traced easily from late anaphase to the reorganizing daughter nuclei, still joined by the drawn-out spindle threads. To explain this it is necessary to assume that the true nucleolus is distinct from this chromatic mass. This, then, would be in line with the second of the explanations offered by Sass for variation in chromosome and reticulum behavior among the several species that he was studying. In support of this hypothesis, that the chromatin is localized or stored in the large "nucleolus-like body" which therefore is to be considered a karyosome from which the chromatin passes into the achromatic framework during pre-meiotic processes, he presents four lines of evidence. Nuclei of *Helicogloea* are too small to permit confirmation. Sass's final statement, that the true nucleolus is a minute body expelled from the chromatin body just before spindle formation, offers a clue to what is evident in *Helicogloea*. As figures 45, 48 (cf. earlier stages also) show, there is a decided decrease in nucleolar size during prophasic periods evident from the first, and after the spindle and chromosomes are definitely formed a minute body is still visible, often some distance removed from the spindle. Later it too disappears. Though this cannot be conclusive without tracing the origin of the reticulum and chromosomes, nevertheless the behavior is suggestive. It seems to occur commonly in many fungi; Wakayama ('30) has promoted similar ideas based on observations of *Aspergillus*.

One of the most curious and striking of aberrations concerns the chromosomes in their passage to the poles. This consistently is irregular, so that from metaphase through anaphase they are scattered from equator to pole, sometimes more or less clumped together. Throughout the literature

this condition is commonly figured (cf. Juel, '98; Ruhland, '01; Kniep, '13; Colley, '18; Bagchee, '25; Wakayama, '30, '30a, '31, '32) not only for basidiomycetes but for ascomycetes as well. Wakayama's extensive works, especially on Agaricaceae, show that this phenomenon is more characteristically present than a regular separation of the chromosomes. His comment that this scattering is due to very different migration velocities for each univalent is the only stated attempt noted in elucidation of the point. He furthermore remarks that in spite of this unusual procedure it nowise follows that the nuclear process is not typical.

Even more striking than this is the behavior of the nuclei in their migration from the parent cells or accessory appendages into the spores. Their extreme chromaticity, attenuation, and loss of form mark the narrow constricted portion through which they must pass just prior to entering the spore as a true sterigma, *sensu* Neuhoff. Such nuclear alteration has been remarked time and time again in the basidiomycetes. Maire ('02) noted that in the heterobasidiomycetes the nucleus passed into the germ tube without change but in moving into the spore through the sterigma it became constricted. It remained for Neuhoff, however, to crystallize the phenomenon into a definite criterion. According to his dictum, cytological in its foundation, a migrating nucleus in passing through a sterigma into a spore assumes just such an elongated form, at the same time giving a chromatin stain reaction. It is tenable then that the long inflated appendages so frequently developed in place of the shorter subulate sterigmata are accessory. This is strengthened still more by the fact that when they reach the surface or a position favorable to spore discharge true sterigmata are formed and the nuclei go through them into the spores in the changed form. That such attenuation is not the result of mechanical constriction due to the size of the sterigma is upheld by the initiation of the condition long before the nucleus reaches that portion of the sterigma (cf. Whelden, '34, on *Tremella*). Spore germination processes are initiated by the production from the primary spore of filaments of varying

length. This in turn develops a true sterigma through which the nucleus moves into the secondary spore, having previously undergone precisely these specified changes. The sterigmata in relation to the spores by all evidence fit Buller's ('22) functional criteria, viz., that "the typical sterigma . . . is . . . an organ for the violent discharge of the spores." The hilum region of the *Helicogloea* spore is well developed. Observation of the actual mechanism of discharge was impossible without fresh material.

A lack of consistency in the terminology employed for the basidiomycetes and the interpretation of their diagnostic structure, the basidium, has long marked the literature, the situation only recently becoming clarified and more constant as careful morphological and cytological studies have brought out true and natural relationships. *Helicogloea* is classified with those basidiomycetes which have transversely septate basidia as opposed to those in which the basidia are vertically divided or those which remain undivided. Historically, the basidiomycetes have been separated into various subdivisions on the basis of basidium morphology, e. g., Hyménomycètes-Hétérobasidiés and Homobasidiés (Patouillard, '87); Proto- and Autobasidiomycetes (Brefeld, '88); and Acrosporeae and Pleurosporeae (van Tieghem, '93). As Gäumann and Dodge *(l. c.)* point out, the first two complements carry a phylogenetic implication and the last is neutral in its connotation. These categories do not comprise identical groups. Van Tieghem considered septum formation an accessory character, the fundamental one for him being the mode of spore insertion, expressed by his terms Acrosporeae and Pleurosporeae. These primary divisions each included both septate and non-septate basidia, which he spoke of as Phragmo- and Holobasidia. Patouillard's groupings have a less artificial basis. Furthermore, he recognized in distinguishing between hetero- and homobasidial groups that the spores of the former usually germinate by the production of secondary spores, whereas those of the latter germinate by mycelia. Such a distinction is a more reliable criterion for primary segregation of the ba-

sidiomycetes than the character of septation. In recent years the use of the terms heterobasidiomycetes and homobasidiomycetes has found acceptance. The heterobasidiomycetes include those forms with secondary spore germination and basidia usually divided in some fashion—the notable exception being the undivided basidia found in the Dacrymycetaceae.

At its earliest inception the basidium of *Helicogloea*, or that of almost any heterobasidiomycete, is not distinguishable from one of a homobasidiomycete at a comparable level of development. Both are usually single, terminal hyphal cells containing a pair of nuclei, descendants of the dikaryon of the sub-terminal cell. From this point onwards the resemblance is diminished in a greater or lesser degree. The true homobasidium remains undivided and develops no accessory parts. Therefore it functions both as zeugite and gonotocont. Variation in some of the heterobasidiomycetes is founded on the separation of these critical stages and consequent modification of morphological patterns.

In *Helicogloea* the initial cell of the basidium complex has been designated as the primordium, a term not inappropriate for the same stage of the homobasidium. From here on the similarity ceases. The subsequent development in the heterobasidiomycetes, and in particular in the genus under discussion, involves the production of distinct and often persistent morphological entities. For *Helicogloea* these take the form of the "sac" and of spore-producing out-growths. Möller called the former "tragzelle," thus merely indicating its position and form with no intimation of its cytological significance. Van Tieghem's terms—probasidium and basidium—have been more widely adopted by later workers. Their connotation does not permit the probasidium as part of the basidium proper and hence introduces a misleading idea of their true relation to the nuclear functions. For this reason they are rejected here. Probasidium, promycelium, and basidium have also been applied to the different parts of the basidium, especially by those who were particularly impressed by the striking resemblances of the group to certain Uredinales.

These terms are rejected on the same basis as the preceding ones.

A cytological interpretation is credited to Wettstein ('11), who emphasized the fact that in the basidium the fusion of two nuclei takes place before the formation of the spores. As Rogers ('34) has stated, the most satisfactory concept of the basidium—for both homo- and hetero-types—is Neuhoff's *(l. c.)*. It is basically cytologic, for he considers the morphological development consequent upon that. According to that author, the mature basidium of the heterobasidiomycetes is composed of a *hypobasidium* which has "germinated" to one or more appendages, the *epibasidia*. This terminology allows adequately for any morphological separation of karyogamy and meiosis. If both these critical events take place in the hypobasidium several epibasidia result, usually four, sometimes eight, consequent upon the number of nuclei produced—as in the Tremellaceae and Tulasnellaceae, respectively; if meiosis is delayed until after germination there is produced, naturally, only one epibasidium. In the latter category are placed the Auriculariaceae, and, among them, *Helicogloea*.

It is a simple matter to reconcile the situation in that genus with this conception. Neuhoff defines the hypobasidium as the "lower, commonly inflated vesicular part of the basidium"; the epibasidium as the "upper, more uniformly tubular part of the basidium." The two parts are in the majority of instances in free communication at all times. If this ground-plan is applied to the behavior in *Helicogloea* all the species resolve neatly into that scheme. That is, the "sac," a lateral expansion of the primordium, is the place where the two nuclei come together and fuse, and therefore it is truly hypobasidial. From the primordium is produced apically a single appendage in which the secondary nucleus undergoes meiosis, the whole eventually elongating and bearing sterigmata and spores. This quite properly is the epibasidium. The two parts are never separated but are at all times in open communication. Even for those species in which the primordium is intercalary this holds. It is evident then that there is nothing in Neuhoff's

criteria to exclude their general application to *Helicogloea*. At first there may seem to be a morphological discrepancy which would prevent their literal adoption. Apparently only those forms with direct germination of the primordium without an accessory part—and the clavate hypobasidium of *H. intermedia* is the only one which answers that description—seem to be completely in harmony with Neuhoff's principles. However, it is only necessary to include for the others the primordial portion as part of the hypobasidium, which therefore is to be described as a hypobasidium with a lateral expansion. In the light of this interpretation the production of the epibasidium is seen to be from the hypobasidium, whatever the constitution of the latter (cf. *H. pinicola*, *H. intermedia*, and *H. Lagerheimi*).

There is good morphological justification for this. The primordium and sac are plainly complementary parts of one structure, and the epibasidium, an outgrowth from either part, is filled with protoplasm at their expense. For reasons of convenience the term primordium is retained to cover the primary phase of the hypobasidium; likewise, the accessory development where karyogamy occurs is referred to as the hypobasidium and in formation is secondary. This is the only example so far known in which there is such an obvious morphological separation of the initial portion of the hypobasidium from that in which karyogamy occurs. One minor deviation from Neuhoff's nuclear scheme must be noted. The secondary nuclei of those Auriculariaceae which he investigated characteristically began migration into the epibasidium during the postsynaptic stage of the first meiotic division. In *Helicogloea* the fusion nucleus contracts to a typical resting condition and then migrates into the epibasidium where it undergoes meiosis. In other words, not only is the hypobasidium divided into two distinct units, a condition not known in other Auriculariaceae, but the nuclear cycle is interrupted by a resting stage—even though it is so short it clearly is only to allow for migration.

The hypobasidium of *Helicogloea* has often been interpreted as a storage organ, an ecological view which Möller inaugu-

rated. His statement that the contents of the hypobasidium would be withdrawn completely and migrate into the developing epibasidium was based on accurate observation, it is true. The idea of a storage organ, unique for this genus, was again expressed by Gäumann ('22). But obviously the time relation between fusion and migration of the secondary nucleus (it contracts and rests only long enough to orient itself for emigration) precludes such an interpretation. One must admit, though, that by virtue of this behavior there is introduced the potentiality for a longer resting period. That the hypobasidium is not a storage organ, in which reserves are held in readiness to produce the epibasidium when favorable conditions so permit, is further substantiated by the observation that as often as not epibasidial germination and elongation occur without any visible augmentation from the hypobasidium. The complementary parts of the hypobasidium are to be construed, then, only in the light of the nuclear cycle.

Within the genus itself there is manifested in basidial characters a progressive sequence from simpler to more advanced forms. Quite obviously, the least developed type would be the clavate form of *Helicogloea intermedia*. The basidium arises as a terminal primordium, becomes swollen to accommodate karyogamy, and then germinates directly to the epibasidium. Here, then, is the simplest type of hypobasidium found in the group, for it consists of only one morphological entity. It is significant that, according to Linder's notations, this type of hypobasidium is the first produced. Since there is a saccate type of hypobasidium in the same species, the production of the clavate type takes on a vestigial aspect. Rogers ('34), going on the assumption that the "primitive auriculariaceous basidium is one with a persistent hypobasidium, clearly distinguishable in all stages," uses as an example the saccate *Helicogloea* hypobasidium showing distal germination. Such behavior is decidedly atypical if it occurs at all. The clavate form is much more acceptable as the primitive type of auriculariaceous basidium, for its hypobasidium is a sharply delimited, persistent organ. Second, there may be placed the

type characteristic of the majority of species, *H. Lagerheimi, H. graminicola,* and *H. caroliniana,* whose basidial primordia likewise are terminal, but which give rise to a secondary part, morphologically distinct. Advance is also indicated by the fact that the clavate type shows no adjustment for proliferation, whereas in the second type there is ample allowance through the subterminal cell development. The remaining species are all marked by this division of the hypobasidium into primary and secondary portions. *H. pinicola* shows an entirely new departure in the intercalary origin of the basidium. But nevertheless the primary portion of the hypobasidium remains the source of germination. (Note that infrequently this pattern is found in the preceding species, too). In the saccate type of *H. intermedia* it is the secondary portion of the hypobasidium which germinates. This possibly represents the highest level, for it suggests a potentiality for a resting stage, and, through encystment, eventual separation. In all types meiosis occurs in the epibasidium.

It is evident that the hypobasidium, and in particular the secondary portion thereof, is the critical organ of the genus. Cytologically this has been shown to be the place of karyogamy followed by contraction to the resting condition. Phylogenetically it offers two lines of interpretation: either reduced or increasing development. Both ideas are extant in the literature as applied to relationships within the Auriculariaceae, and between them and other groups.

In the Auriculariaceae, Neuhoff constructs a phylogenetic scheme involving both tendencies. He considers the genus *Iola* the crucial one, for there the basidium arises from a terminal cell and is divided into hypobasidium and epibasidium, the former remaining distinct. This gives rise to two main lines, one showing reduction in differentiation of the hypobasidium, the other increasing emphasis. The first of these is subdivided into a series in which reduction eventually leads to complete suppression, as illustrated by the genus *Helicobasidium;* and another in which the hypobasidium is present though it may be reduced to a vestigial condition, as read in the series

Achroomyces, Tjibodasia, and *Auricularia.* The second line emphasizes the hypobasidium through the thickening of its walls and the introduction thereby of a resting period. This plan of development is illustrated by the genera *Cystobasidium* and *Septobasidium.* In the latter there are especially critical characters, for the thickening of the walls in some species is so advanced that in view of that and a consequent resting period, the hypobasidium is known as a sclerobasidium. This condition then is strongly like that of the rust teliospore. Indeed, the only real basidial difference between this and such primitive rusts as *Gallowaya* is the catenulate nature of the teliospores of the latter. Consequently, Neuhoff sees here a direct link to the Uredinales through such a genus, for instance, as *Uromyces,* whose teliospores are borne singly on the hyphae. Hence the sclerobasidium (encysted hypobasidium) and the teliospore are homologous.

That there is a close relationship between the Auriculariaceae and the Uredinales is argued by more than mere analogy. Cytologically it is demonstrable that the teliospore and hypobasidium are strictly homologous; likewise the promycelium and epibasidium; sporidia and basidiospores. Möller was the first to comment on this, but to him the hypobasidium of *Helicogloea* and the teliospore were homologous because both were the assembling point of materials necessary for basidium production. Since in the former no resting period was involved there was no need for a heavy wall. This ecological interpretation has since been replaced by the more substantial cytological one. Moreover, the ecological argument lost credibility as investigations revealed that the teliospore is not necessarily designed for wintering over, for there are rusts whose teliospores germinate at once. Again there are rusts whose teliospores are thin-walled, just as in *Septobasidium* of the Auriculariaceae, where the development passes from species with thin-walled, non-resting hypobasidia—hardly more than a stage in development—to thick-walled, persistent forms which rest (winter-over) before germinating. Such striking parallelisms emphasize the closeness of the auriculariaceous fungi and the rusts.

The majority of writers have adhered to de Bary's ('84) tenets that the Uredinales constitute the most primitive of the basidiomycetes. Recently Linder ('29) and Pady ('33) have stressed this by deriving the auriculariaceous forms from the Uredinales. Thus Linder, starting with the Uredinales would, by reduction of the hypobasidium, lead the developmental line through *Septobasidium* and *Cystobasidium* to *Iola* with secondary branches to *Helicogloea* and *Auricularia*. *Septobasidium* holds the closest connection with rusts, since it is parasitic. It stands as a source of further development by reason of two different hypobasidial expressions: thick-walled forms which are adapted for wintering-over and thin-walled forms which have no resting period prior to germination and which really are no more than a stage in development. The latter affords a link to *Iola*, a parasitic form whose thin-walled hypobasidia likewise germinate at once. *Helicogloea* has lost both the parasitic habit and the thickened hypobasidia. *Auricularia* is even more reduced, for there the hypobasidium is represented only as a stage. Specifically, by virtue of the clavate type of hypobasidium, Linder regarded *Helicogloea intermedia* as the form transitional between the rusts and *Iola*. To achieve such a step from rusts to auriculariaceous species, there must be postulated the concomitant factors—reduction of aeciospores to binucleate conidia, development of an extensive fruiting body, and finally a change from parasitic to saprobic habit.

In opposition to such a derivative scheme it may be contended that it is more logical to derive aeciospores from binucleate conidia. This Neuhoff has expressed when he homologized the aeciospores with diploid conidia known in the Auriculariaceae and the pycniospores with haploid conidia. Thus the variety of secondary spore forms in the rusts cannot be said to constitute an obstacle to relationships between the two groups in light of the occurrence of both haploid and diploid conidia in the Tremellales. Especially does the argument of transition from parasitic to saprobic habit seem untenable. As Janchen ('23) has remarked, an attempt at

phylogenetic elucidation must be not only morphologically acceptable, but ecologically comprehensible as well, if it is to be satisfactory. And exactly in its ecological expression does this derivation of Auriculariaceae from Uredinales seem to fail. The high specialization attained by the rusts much more clearly marks them as an end term of a series than as a derivation point. The reverse derivation—the rusts from auriculariaceous forms—is not inconceivable ecologically when one considers the numerous examples of parasitic species of Auriculariaceae.

The foregoing discussion is intended only to summarize some of the more outstanding phylogenetic interpretations in the literature. In view of the greater defensibility of the derivation of the rusts from the Auriculariaceae, that plan is upheld. *Helicogloea* is regarded therefore as representing a simple auriculariaceous type showing tendencies which could lead to the devolpment of an independent hypobasidium and hence to a true teliospore.

Summary

1. *Helicogloea* Pat. takes precedence over *Saccoblastia* Möll. with corresponding transfer of recognized species. *Saccoblastia ovispora* and *S. sebacea* are both regarded as synonyms of *Helicogloea Lagerheimi* Pat.

2. In *Helicogloea Lagerheimi* Pat. the basidial primordium is a two-nucleate hyphal cell, producing a lateral expansion.

3. The dikaryon migrates into the expanded portion of the hypobasidium where fusion takes place.

4. Following contraction to the resting condition the secondary nucleus migrates through the primordial portion into the elongating epibasidium, which has arisen apically from the primordium.

5. The two meiotic divisions always take place in the enlarged portion of the epibasidium. The latter segments into four cells and each segment produces a sterigma and a spore or an accessory appendage bearing a sterigma and a spore.

6. Spore germination is by repetition.

7. Morphological development is various in relation to the nuclear cycle; it has been found to correspond to one of three patterns, designated as I, II, and III.

8. The basidium complex is composed of hypobasidium and epibasidium, *sensu* Neuhoff. The former comprises two morphologically distinct parts: the primordium, or primary portion, and its lateral expansion, the "sac," or secondary portion.

9. Within the genus there is seen a progressive sequence from simpler to more advanced forms of basidia, the clavate hypobasidium of *H. intermedia* being interpreted as the most primitive.

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Explanation of Plate

PLATE 7

Helicogloea Lagerheimi

All figures have been drawn with the aid of an Abbé camera lucida. Magnifications are given for each figure.

Figs. 1–13 inclusive show the morphological development of the basidium from the primordial stage through the mature basidium with spores. The nuclear conditions are representative only for these particular examples. All figures × 1100.

Fig. 1. Enlarged terminal hyphal cell, the basidial primordium, with two nuclei.

Fig. 2. Production of the hypobasidium as a lateral expansion of the primordium.

Fig. 3. Hypobasidium enlarging, the nuclei moving toward the enlarging secondary portion of the hypobasidium.

Fig. 4. Mature hypobasidium with two nuclei in it.

Fig. 5. Mature hypobasidium with fusion nucleus; epibasidium arising at the apical end of the primordium.

Fig. 6. Nucleus migrating through the primordium; hypobasidium collapsing as the protoplasm withdraws; epibasidium elongating.

Fig. 7. Continued elongation of the epibasidium and withdrawal of protoplasm from the hypobasidium.

Fig. 8. Nucleus in enlarging epibasidium; hypobasidium entirely empty.

Fig. 9. Enlarging epibasidium.

Fig. 10. Epibasidium bending over.

Fig. 11. Epibasidium two-septate, following the first meiotic division; protoplasm withdrawn partly from lower portions.

Fig. 12. Epibasidium three-septate, 4-nucleate; budding sterigmata; the lower portions of the epibasidium collapsing, completely drained of protoplasm.

Fig. 13. Mature basidium with spores in place.

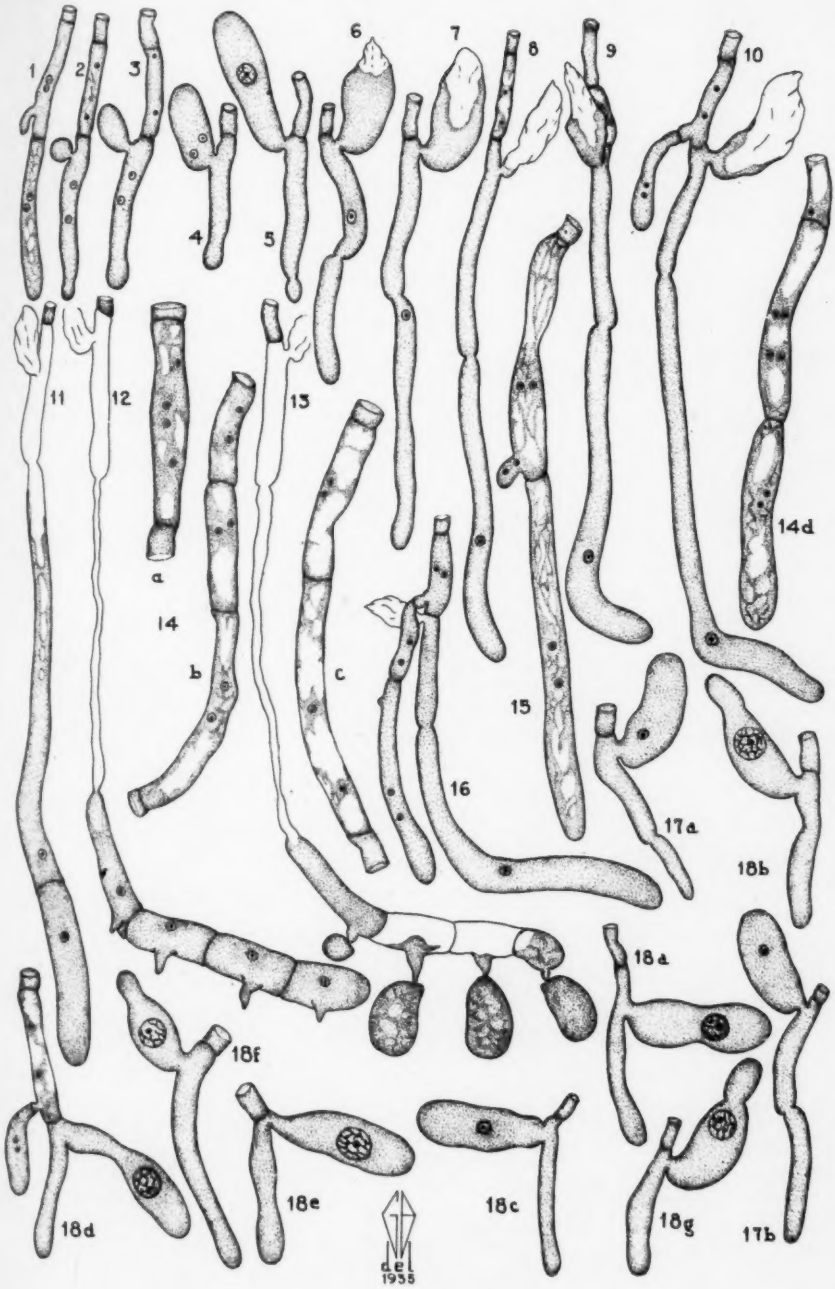
Fig. 14. Hyphal cells. a, d, young cells in which four nuclei are present, for the wall is not yet formed; b, c, older cells with increased reduction of protoplasm. × 1865.

Fig. 15. Origin of a branch from a subterminal cell; the terminal cell constitutes a basidial primordium. × 1865.

Fig. 16. Basidial proliferation. (See also fig. 18d.) × 1100.

Fig. 17. Developing epibasidia showing a crimp between the epibasidium and hypobasidium. × 1100.

Fig. 18. Variations in hypobasidial form. a, b, constricted hypobasidia; c, horizontal hypobasidium; d, e, pendent hypobasidia; f, g, extreme distal constriction. × 1100.



BAKER — HELICOGLOEA

Explanation of Plate

PLATE 8

Helicogloea Lagerheimi

Fig. 19. Composite view of the fructification on a wood substratum as seen in section. × 464.

Fig. 20. Scales to illustrate the proportion of fruiting and hyphal regions in differently developed fructifications. × 100.

Fig. 21. a, b, basidia arising from lateral branches. × 1100.

Fig. 22. Origin of epibasidium prior to appearance of hypobasidium. × 1865.

Fig. 23. a, b, c, irregular, lateral production of epibasidia. Surface level of the fructification marked. × 1100.

Fig. 24. Type I of nuclear migration from the hypobasidium. × 1100.

Fig. 25. Type II of nuclear migration from the hypobasidium. a, typical form; b, unconstricted type.

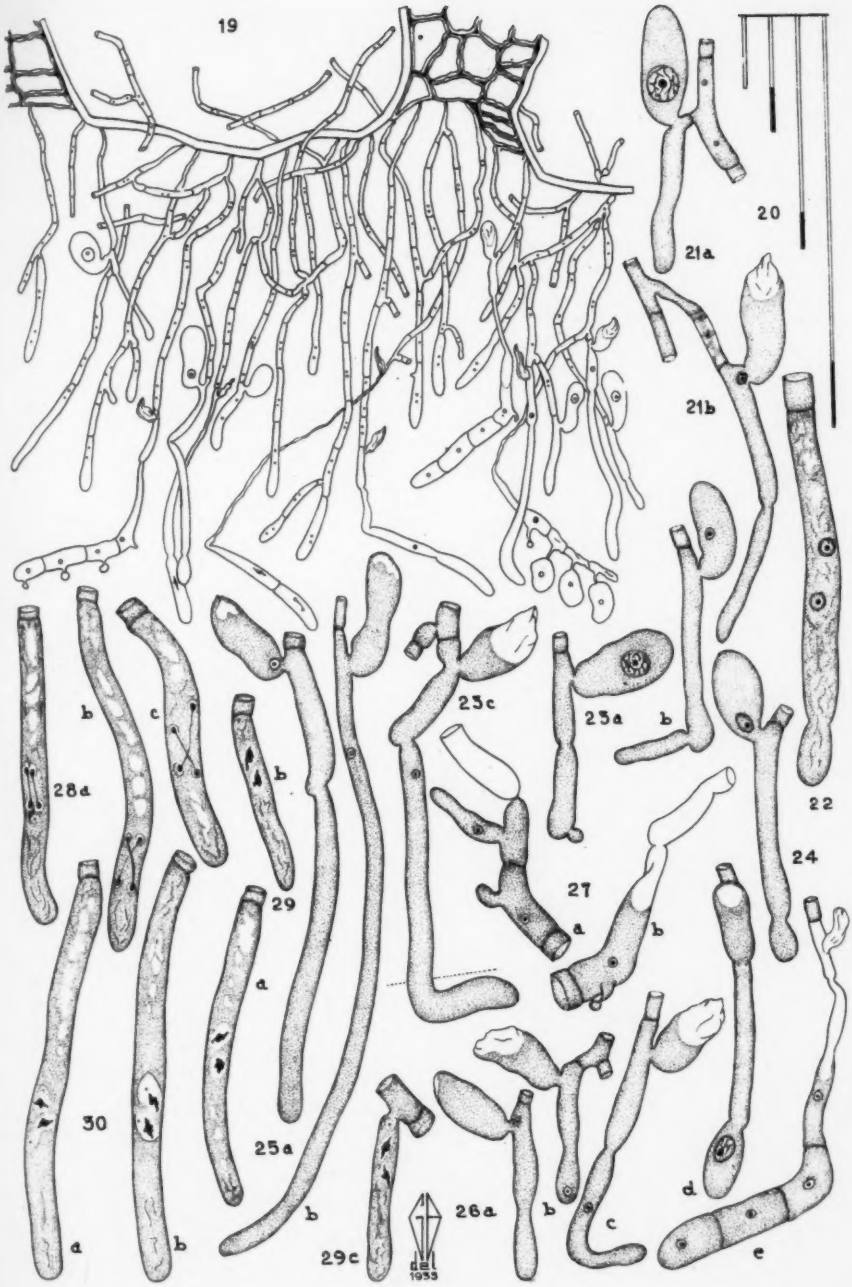
Fig. 26. a, Type III of nuclear migration. b, c, d, e, subsequent development.

Fig. 27. a, deep constriction and septum at the base of the fourth segment of the epibasidium; b, constriction without a wall. × 1100.

Fig. 28. a, conjugate mitosis, with nearly parallel orientation; b, c, crossed spindles. × 1865.

Fig. 29. a, b, c, mitotic division figures indicating future crossed orientation of the spindles. In *c* a remnant of the nucleolus is visible. × 1865.

Fig. 30. Mitosis. a, membranes completely broken down, nucleoli still visible; b, nuclear membranes present in part, nucleoli distinct. × 1865.



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Explanation of Plate

PLATE 9

Helicogloea Lagerheimi

Fig. 31. a, basidial primordium with the basal wall just formed; b, older primordium with hypobasidium and epibasidium both developing. × 1865.

Fig. 32. A series to show the increase in size from the initiation of the primordium through the hypobasidium production. × 1865.

Fig. 33. Origin of the hypobasidium. (See also fig. 31b.) × 1865.

Fig. 34. a, b, nuclei in close association previous to migration into the hypobasidium. × 1865.

Fig. 35. Lag of second nucleus in passage into hypobasidium. × 1865.

Fig. 36. a, b, nuclei moving into hypobasidium. In *b* the first nucleus is prematurely enlarged prior to fusion. × 1865.

Fig. 37. Nuclei in hypobasidium previous to fusion. Note effect of streaming protoplasm. × 1865.

Fig. 38. Nuclear fusion. a, nuclei touching, nuclear membranes still intact; b, nuclei as in *a*, but enlarged and showing a reticulate nature; c, nuclear membranes broken down and the contents mingling; d, nuclei fusing, one larger than the other with slightly more pronounced reticulations; e, nuclei of different sizes corresponding to their development, both deeper in the hypobasidium than usual. × 1865.

Fig. 39. Fused nuclei with an indentation between the two still apparent. Within the common membrane there is an advancing net development. × 1865.

Fig. 40. a, b, c, stages in nucleolar fusion. × 1865.

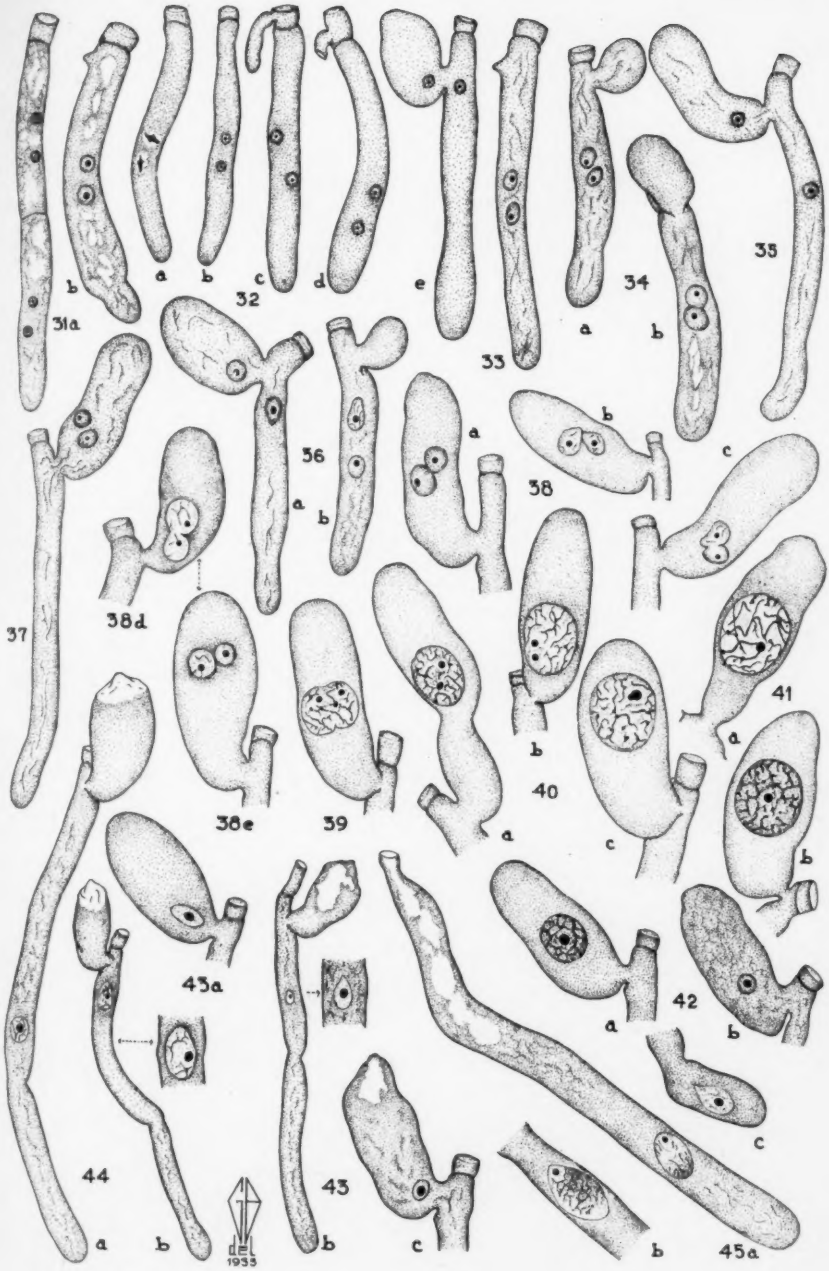
Fig. 41. a, post-fusion nucleus, with strongly developed network; b, post-fusion nucleus with prominent chromatic beads scattered on the threads. × 1865.

Fig. 42. a, nucleus contracting, the network still pronounced; b, nucleus in resting condition, completely contracted. × 1865.

Fig. 43. a, b, c, examples of nuclear passage from hypobasidium; a, detail of fig. 24; c, detail of fig. 25. × 1865.

Fig. 44. Variation in the initiation of prophasic activity. a, nucleus enlarged, reticulate. × 1865. b, nucleus still in primordial portion. × 1100 with detail × 1865.

Fig. 45. Prophase in sporogenous part of the epibasidium. a, nuclear membrane intact, nucleolus peripheral, network appearing opposite pole of nucleolus; b, the network appearing slightly polarized; c, faint reticulations in enlarged nucleus from epibasidium of Type III. All × 1865.



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Explanation of Plate

PLATE 10

Helicogloea Lagerheimi

Fig. 46. Prophase of first meiotic division. The nucleus is elongate, conforming to the shape of the epibasidium. × 1100 with detail × 1865.

Fig. 47. Early prophase with chromatic beads on the threads. × 1865.

Fig. 48. Prophase. a, nucleolus, slightly diminished in size, lying at anterior pole and the threads grouped at the opposite pole. × 1865; b, nucleolus still prominent, the threads less massed and central in position. × 1100 with detail × 1865; c, later prophase, the nucleolus at the periphery, the chromatin densely massed. × 1865.

Fig. 49. Early prophase from an epibasidium with a development corresponding to Type III. × 1865.

Fig. 50. Anaphase. a, intranuclear spindle with scattered chromosomes. Remnants of the nucleolus are visible within the membrane and centrosomes are present at the poles. b, nuclear membrane present in part; nucleolus sharp; c, d, e, membranes broken down and spindles free in cytoplasm; the nuclei have disappeared. × 1865.

Fig. 51. Late anaphase. a, nuclear membrane discernible in part, the nucleolus distinct in the cytoplasm; b, membrane entirely gone, chromosomes massing together as they move to the poles; c, spindle contracted, chromosomes clumped, massing at the poles. The clearer areas at the poles denote the position of the daughter nuclei. × 1865.

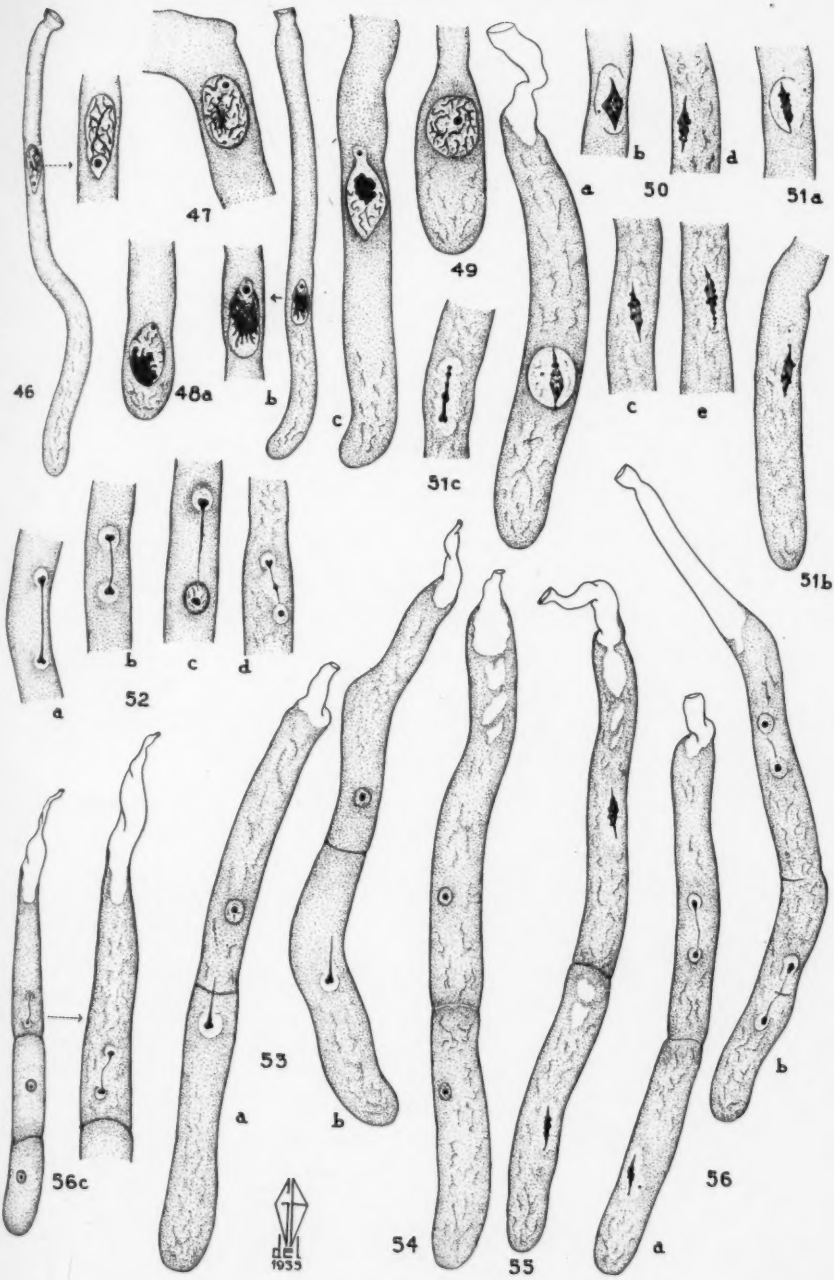
Fig. 52. Telophase. c, chromosomes massed at the poles, the two groups still connected by distinct fibers, daughter nuclei organizing; b, daughter nuclei more prominent and the fiber connections diminishing; c, lower nucleus nearly completely organized, the upper not so advanced; fibers prominent in upper region where they are still attached to the nucleus; d, darker area on connecting fibers indicative of wall formation. × 1865.

Fig. 53. Two-nucleate stage. a, upper nucleus completely organized, lower nucleus still showing fiber connections; the wall is just coming in; b, wall just formed and the nuclei farther apart than in a. × 1865.

Fig. 54. Two-nucleate stage. The nuclei are reorganized but the wall is not yet formed. A clearer zone shows its future position. × 1865.

Fig. 55. Second meiotic division. Both spindles are in anaphase, the nuclear membranes have disappeared as well as the nucleoli. × 1865.

Fig. 56. Variation in the synchronization of the second meiotic division. a, lower nucleus in anaphase, nucleolus distinct; upper nucleus in telophase. × 1865; b, lower nucleus in telophase with fiber connections still visible and the wall coming in; upper nucleus nearly completely organized, the fibers still just discernible. × 1865; c, daughter nuclei of lower segments completely formed with a wall between; basal nuclei still in telophase with connecting fibers between. × 1100 with detail × 1865.



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EXPLANATION OF PLATE

PLATE 11

Helicogloea Lagerheimi

Fig. 57. Four-nucleate, four-septate epibasidium, two of the walls having just been formed. × 1865.

Fig. 58. a, fourth segment of epibasidium with a basal septum; b, fourth segment with protoplasmic remnants simulating a septum. × 1865.

Fig. 59. Segment from an epibasidium with a nucleus showing two nucleoli. × 1865.

Fig. 60. Budding sterigmata. The wall is not completely formed between the two end cells. × 1865.

Fig. 61. Sterigma with spore developing on the second segment of the epibasidium. × 1865.

Fig. 62. Sterigmatic formation. In segments 2 and 3 the nuclei are changing form preparatory to passing into the spores. × 1865.

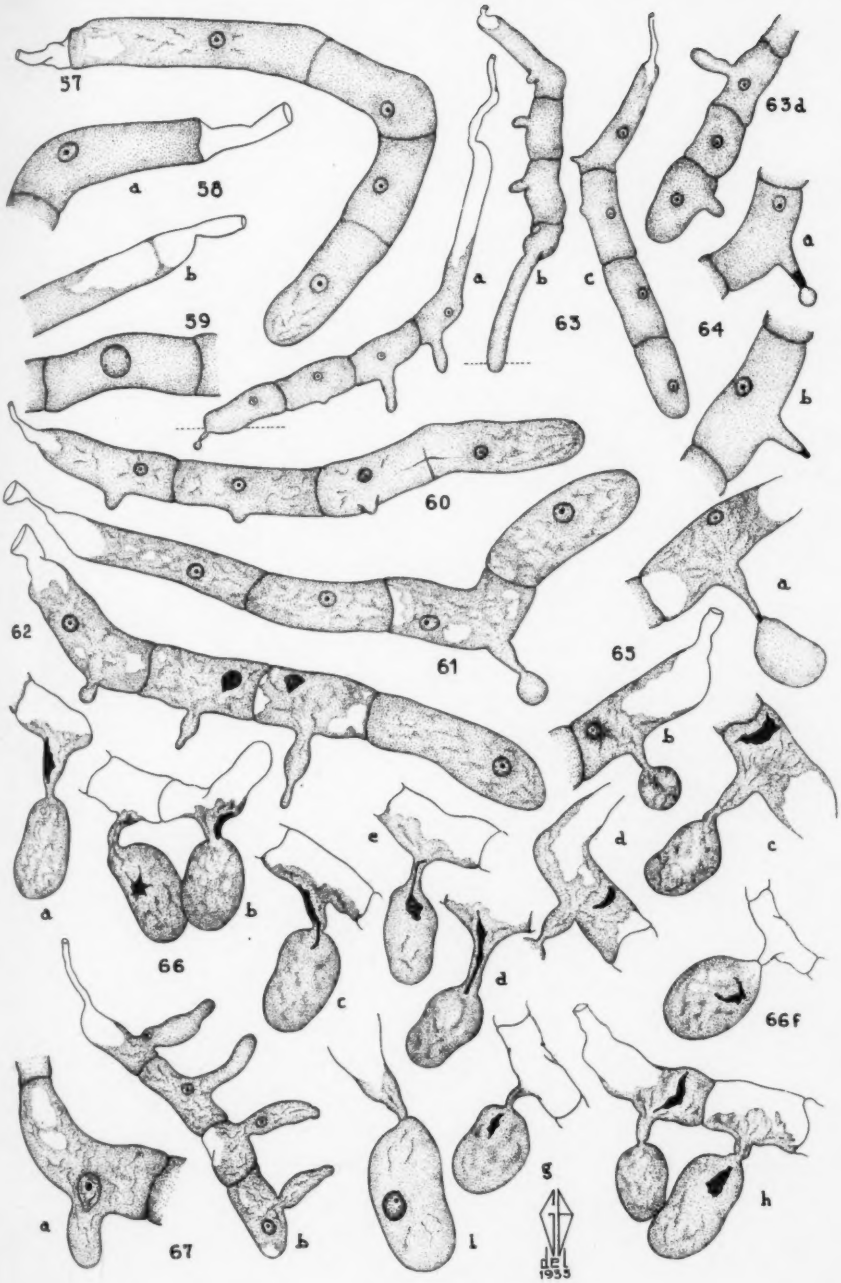
Fig. 63. Variations in sporogenous portion of the epibasidium. a, apical segment with typical sterigma; lower segments developing longer appendages; surface level marked; b, apical segment producing long appendage; surface level marked. Note the bend in the basal segment. c, sterigmatic initiation in the fourth segment; d, sterigmata forming on opposite sides. All × 1100.

Fig. 64. a, b, developing sterigmata with dark staining tips. × 1865.

Fig. 65. Nuclear behavior preparatory to passage into the spores. a, nucleus normal, spore about half developed. Note that the dark-staining tip is still apparent; b, nucleus becoming deeply stainable; protoplasm withdrawing into the developing spore; c, nucleus dark-staining, irregular, attenuate; d, nucleus a homogeneous dark mass. All × 1865.

Fig. 66. Nuclear passage into the spore. a, nucleus in sterigma; b, nucleus of end segment, in sterigma; second segment completely drained of protoplasm and nucleus reorganizing in the spore; c, d, nuclei much drawn out in passage through the sterigmata; e, nucleus mostly within the spore; f, g, nuclei not yet reorganized in the spores; h, one nucleus reorganizing in the spore, nucleus of the basal segment ready to migrate; i, nucleus reorganized in the spore. All × 1865.

Fig. 67. a, nucleus moving into a long appendage without change in form. × 1865; b, sporogenous portion of an epibasidium, all segments of which have produced long appendages. × 1100.



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EXPLANATION OF PLATE

PLATE 12

Helicogloea Lagerheimi

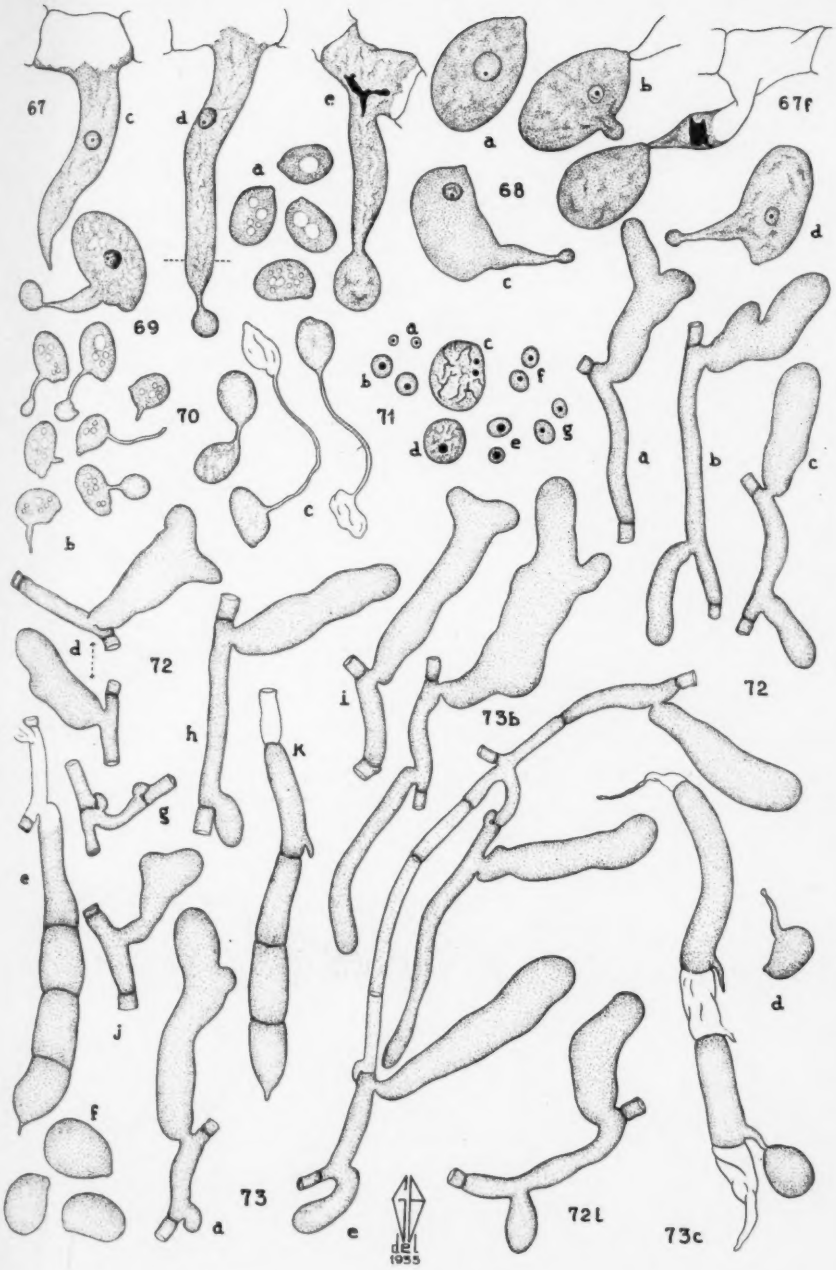
Fig. 67. c, d, formation of sterigmata and spores at the ends of long appendages. Nucleus unchanged at this stage. Surface level marked in d; e, f, nuclei prematurely changed for passage into sterigmata and spores. All × 1865.

Fig. 68. a, typical spore; b, spore germinating while still attached to the sterigma; c, d, germination by repetition. × 1865.

Fig. 69. Nucleus changing for passage through germ tube and sterigma into secondary spore. × 1865.

Fig. 70. Germination of spores in hanging-drop cultures. a, stages prior to germ tube production, 24 hours; b, production of germ tubes and secondary spores, 24–48 hours; c, protoplasm withdrawn into secondary spores, 72 hours. All × 734.

Fig. 71. Nuclei showing varying sizes through the cycle and corresponding nucleolar volumes. All from the same section and × 1865. a, dikaryon from terminal hyphal cell; b, dikaryon prior to migration into hypobasidium; c, fusion nucleus in hypobasidium; d, post-fusion, nucleus contracting, nucleolar volume obviously increased; e, nuclei from migrating stages after fusion; f, nuclei from 4-celled stage of epibasidium, prior to sterigmatic formation; g, nuclei from typical spores.



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Explanation of Plate

PLATE 13

Fig. 72. *Helicogloea pinicola*, No. 20124. a, forked hypobasidium; b, budding epibasidium sub-apical in position; c, budding epibasidium slightly sub-apical in position; d, typical hypobasidia; e, septate epibasidium; f, spores; g, clamp connections on the hyphae.

No. 20555. h, slightly constricted hypobasidium; i, j, forked hypobasidia; k, septate epibasidium with a fourth septum present; l, sub-apical epibasidium. All figures × 434.

Fig. 73. *H. pinicola* f. *alniviridis*. a, origin of epibasidium, hypobasidium forked; b, irregular hypobasidium, typical epibasidium; c, septate epibasidium, two segments of which are already discharged; d, germinating spore; e, proliferation of basidia. All × 434.

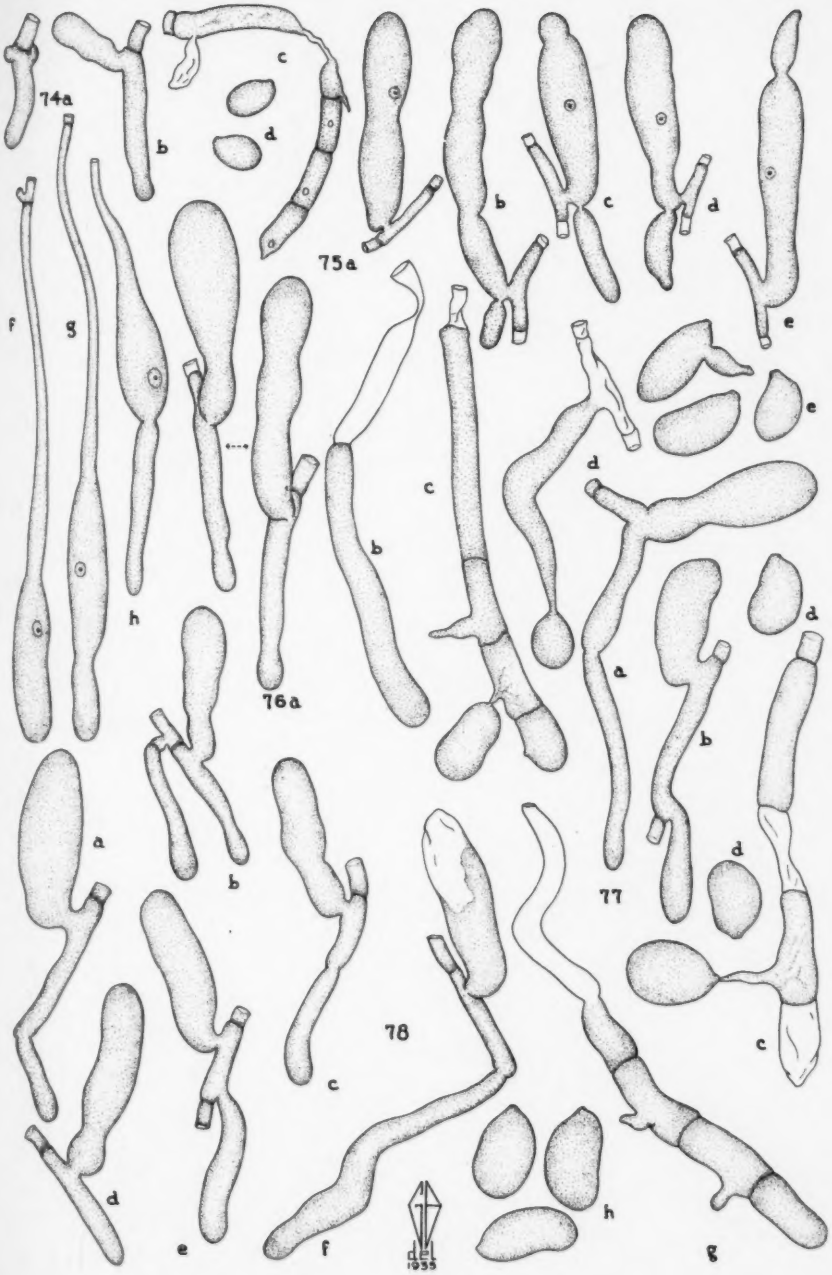
Fig. 74. *Helicogloea graminicola*. a, origin of the hypobasidium; b, mature hypobasidium; c, septate epibasidium. Note that all the protoplasm has not withdrawn from the hypobasidium. d, spores. All × 434.

Fig. 75. *Helicogloea intermedia*. a, constricted hypobasidium; b, origin of the epibasidium, hypobasidium much constricted; c, basal constriction of hypobasidium; d, typical origin of epibasidium; e, proximal production of epibasidium or basal constriction; f, g, h, stages in the development of the clavate type of hypobasidium. All × 734.

Fig. 76. *Helicogloea caroliniana*. a, variation in the hypobasidium; b, epibasidium; c, epibasidium with developing sterigmata and spore; d, long appendage with sterigma and spore; e, mature spores. All × 1100.

Fig. 77. *Helicogloea Lagerheimi*, No. 42262, marked as var. *vulgaris*. a, constricted hypobasidium, typical epibasidium of terminal origin; b, intercalary origin of the basidium; c, septate epibasidium with four septa; two segments have already discharged their spores; d, spores. All × 1100.

Fig. 78. *Helicogloea Lagerheimi*, Rick's material. a, typical hypobasidium and epibasidium; b, c, d, variously constricted hypobasidia; e, intercalary origin of basidium; f, developing sporogenous portion of epibasidium; g, epibasidium developing sterigmata; h, spores. All × 1100.



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Explanation of Plate

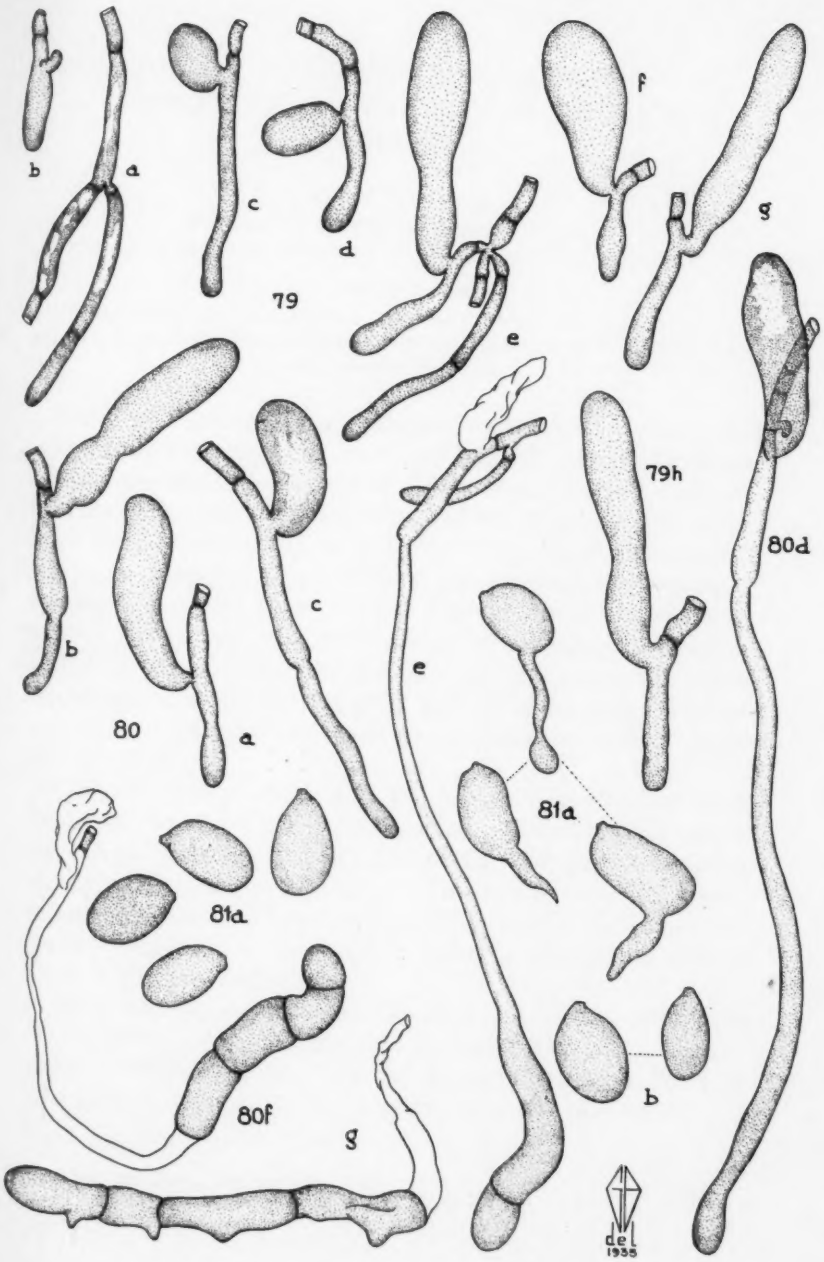
PLATE 14

Helicogloea Lagerheimi, type material

Fig. 79. a, terminal primordium and hyphae; b, slightly older primordium with origin of hypobasidium; c, hypobasidium more advanced; d, hypobasidium unusually far forward on primordium; e, large, constricted hypobasidium; f, short broad hypobasidium; g, long slender hypobasidium; h, unusually long hypobasidium. All × 1100.

Fig. 80. a, epibasidium just distinguishable; b, epibasidium slightly more advanced; c, epibasidium elongating, hypobasidium emptying; d, epibasidium prior to thickening of sporogenous portion; e, sporogenous portion two-celled; f, sporogenous portion four-celled; g, sporogenous portion four-septate with sterigmata. All × 1100.

Fig. 81. a, four spores; b, three germinating spores; c, one large, one small spore. All × 1100.



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MORPHOLOGY, PHYSIOLOGY AND CYTOLOGY OF SYRINGOSPORA INEXORABILIS (MONILIA INEXORABILIS)

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While we were studying pathogenic species of *Zymonema*, Mazza and Palamedi ('32) described *Monilia inexorabilis* from a fatal case of blastomycosis of the skin and mucosa. His illustrations showed an imperfect stage which closely resembled some seen in lesions and in cultures of species of *Zymonema*. Dr. Mazza kindly sent us a culture, and although the organism has been grown for four years on a great variety of media, it has not shown any trace of sexuality or ascospore formation such as is common in *Z. dermatitidis* and *Z. capsulatum*. The study was resumed early in 1934, and sufficient morphological and cytological data have now accumulated to warrant their presentation.

The patient was a Spaniard, 53 years of age, who had lived for 21 years in the Chaco of Argentina. Ulcerating lesions on the buccal mucosae were diagnosed as blastomycotic, and a biopsy confirmed the diagnosis. The lesions were of a progressive nature and involved the whole respiratory tract, becoming generalized. A complete autopsy established this as "true" blastomycosis.

We gratefully acknowledge Dr. Salvador Mazza, Jefe de la Misión de Estudios de Patologia Regional Argentina, for cultures of this organism, and the Science Research Fund of Washington University for a grant in aid of this work.

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METHODS OF TECHNIQUE

For a morphological study of the organism, mounts of mycelium were made in Amann's lacto-phenol and 0.5 per cent cotton blue. Distilled-water mounts were also favorable because their uniform staining did not distort the material. Several dyes, such as 1 per cent aqueous crystal violet to the desired intensity in glycerine, methylene blue, neutral red, and iodine green, were used to bring out granulation. The life cycle of the fungus was observed from hanging-drop preparations made with lactose broth, nutrient broth (meat extract), and 2 per cent proteose peptone. Iodine potassium iodide (saturated solution) was used for studying the glycogen content of the cells and the possible chondriosomes, as advocated by Guilliermond; neutral-red and methylene blue, for studying volutin or metachromatic material. A pinch of benzidine sulphate added to a water mount of organisms showed the so-called "dancing bodies." Osmic acid 2 per cent, and platinic chloride 5 per cent, demonstrated fat and lipoidal substances.

For a study of the nuclear phenomena, agar slants of the culture were killed and fixed with Hermann's fluid and embedded in celloidin (Moore, '33). Sections were then cut to a thickness of 10 μ, and stained with Haidenhain's iron-alum haematoxylin with no counterstain. After being cleared in benzol they were mounted in Canada balsam. When embedded in celloidin the material has little or no shrinkage and is cleared very nicely.

DESCRIPTION

The organism was obtained on a Sabouraud's glucose agar slant. In the host the parasite exists as a single or budding yeast-like cell which may very easily be confused with that found in blastomycosis. When cultivated on an artificial substrate, the cells send out germ-tubes or elongate (pl. 15, figs. 1–4) to produce hyphae of cells which are formed by the development of cross-walls (figs. 5–6). These vary from 2 to 5 μ in diameter according to the medium on which grown. On several media, however, the yeast-like budding cells may per-

sist, or at least predominate for a variable length of time (figs. 9, 25), and measure from 2 to 17 μ in diameter. Wort agar is especially adapted for the continuance of the yeast stage, and blood agar and serum media, to a certain degree (figs. 24–25).

The continued growth as a saprophyte results in morphological changes which help in the classification of these yeast-like fungi. The elongated forms developing from the germinating cell vary both in diameter and length. On media with beef extract near the neutral point or with a high pH, the filaments generally are long and have a small diameter (figs. 10–11, 23). On media with a low pH, as Raulin's malt extract, Czapek's, or wort agar, the cells are shorter and have a greater diameter (figs. 8–9, 12–13, 16–17). On wort agar (fig. 12) the organism becomes very large, spherical, and thick-walled. This peculiar condition is noticed to a lesser degree on malt extract agar (fig. 9), which is a modification of wort agar.

A verticillate or dendroid type of growth is rather common on most media for this fungus, but definitely characteristic on Raulin's solution (figs. 16–17). The cells of the main axes of the structure (fig. 17) are somewhat longer than those which bud off from them, the blastospores. The blastospores develop along with the hyphae as single or multiple cells, terminally (figs. 16–17, 20, 22), laterally (figs. 8, 10, 18–23), or at the point of division of two elongated cells of the filaments (fig. 17). They may either be spherical to ovoid or subpyriform to pyriform, and measure approximately 3–6 μ in diameter, usually 5 μ or more. These cells are uninucleate and are capable of developing new colonies if allowed to germinate under favorable conditions.

Besides the structures mentioned, there are also large terminal, clavate or obovate cells measuring 9–15 μ in diameter or long axis. They are double-contoured (thick-walled) structures and are found as modified cells on malt extract, glycerine, yeast dextrose, nutrient blood agar, and even in serum. Lanceolate cells 9–15 μ in long axis also occur, usually singly or budding, and are modifications of the spherical or ovoid cells found on various substrates. Another structure noted is the

thick-walled cell or cells sometimes found at the base of a verticillate growth (fig. 16). These are the germ cells which develop a thickened wall in much the same manner as would the root of a plant.

The nuclear phenomena of development will be considered under cytology.

CULTURAL CHARACTERISTICS

The organism when received was growing on a Sabouraud's agar slant. Subcultures from this growth were made on a number of different media, varying in hydrogen ion concentration, protein or protein decomposition products as peptones, and in the amounts of carbohydrate and nitrogen. All cultures were grown at room temperature, approximately 22° C., on the following media arranged in the order of increasing pH.

Raulin's solution (pH 4.1).—Sediment of growth on bottom of flask. Culture monilioid. Groups of budding yeast-like cells, double contoured, 5–12 μ in diameter. Filaments 2–5 μ in diameter; blastospores 3–5 μ in diameter; mycelium verticillate.

Czapek's Agar (pH 4.4).—Colony approximately 3.5 cm. in diameter after 40 days. Mycelium mostly submerged in agar, tending to form coremia of elongated cells approximately 3 μ in diameter. Spherical and ovoid, yeast-like cells 3–17 μ in diameter; racquet and verticillate mycelium; blastospores of varying proportions, spherical to ovoid.

Malt Extract Agar (Difco, pH 4.6).—Colony approximately 3.5 cm. in diameter after 40 days. Mycelium submerged in medium, with a general appearance similar to that on Czapek's agar. Color creamy-buff. Hyphae approximately 3 μ in diameter; blastospores approximately 6 μ in diameter. Budding cells 6–12 μ in diameter on surface of medium, with branching, coremioid mycelium. Terminal clavate, obovate to lanceolate cells approximately 12–15 μ in long axis.

Wort Agar (Difco, pH 4.8).—Colony approximately 3.5 cm. in diameter after 40 days. Color light coffee-brown. Culture

shows excrescences. Verticillate growth of cells 3–15 μ in diameter, the large spherical cells having a thick capsule. Budding cells present. Filamentous mycelium approximately 3 μ in diameter, in medium, with some elongated cells and blastospores approximately 4 μ in diameter. Terminal obovate to lanceolate cells present.

Sabouraud's Agar (pH 5.6).—Colony approximately 5 cm. in diameter after 40 days. Most of culture submerged, portion on surface approximately 2.5 cm. in diameter with a heaped-up growth in center. Growth excrescences and radiating ridges to the exposed periphery evident. Color dark creamy-buff. Submerged mycelium filamentous, 2–5 μ in diameter, branching, coremioid and somewhat verticillate. Blastospores approximately 6 μ in diameter. Yeast-like, budding, also germinating, cells 3–12 μ in diameter. Obovate or clavate terminal cells on submerged filaments. Surface growth of yeast-like cells.

Corn-Meal Agar (Difco, pH 6.0).—Colony approximately 3.5 cm. in diameter after 40 days. Culture almost entirely submerged with a surface growth approximately 0.7 cm. in diameter. Color dull creamy-buff. Macroscopic appearance similar to that on Czapek's agar. Surface growth of yeast-like budding cells, same as on Sabouraud's agar. Filaments much thickened, branching, coremioid; blastospores thick-walled, approximately 5 μ in diameter.

Potato-Dextrose Agar (pH 6.2).—Colony approximately 3.5 cm. in diameter, with an irregular periphery. Growth on surface of yeast-like, budding cells approximately 2–10 μ in diameter. Submerged mycelium of filamentous forms with hyphae approximately 3 μ in diameter; blastospores approximately 6 μ in diameter. Mycelium verticillate as in other substrates.

Potato-Dextrose Broth (pH 6.2).—Sediment at bottom of flask. Mycelium similar to that on the agar.

Nutrient Agar (Difco, pH 6.6).—Colony moist and irregular with growth partially submerged in agar, approximately 2.5

cm. in diameter. Cells on surface mostly spherical to ovoid, budding, up to 12 μ in diameter. Large cells 15 μ in diameter. Large spherical cells within medium and smaller ovoid to spherical forms on surface.

Nutrient Broth (Difco, pH 6.7).—Sediment at bottom of flask. Yeast-like cells as on the above medium, with filamentous forms monilioid in appearance, approximately 3 μ in diameter. Blastospores subpyriform, 5 μ in diameter.

Lactose Agar (Difco, pH 6.8).—Colony approximately 2 cm. in diameter. Submerged mycelium a verticillate, coremioid growth similar to that in Czapek's agar. Growth on surface moist and cream-colored, with many budding, yeast-like cells, spherical and ovoid, 3–12 μ in diameter. Filaments elongated, 2–3 μ in diameter, monilioid and blasto-dendroid, verticillate; blastospores pyriform, subclavate or ovoid, in varying proportions.

Glycerine Agar (nutrient agar plus 6 per cent glycerine, pH 7.0).—Colony approximately 5 cm. in diameter, both submerged in the agar and on the surface. Growth on surface approximately 3.5 cm. in diameter with an irregular periphery, dull cream in color. Central region of erupted, vesicular-like outgrowths. Yeast-like cells spherical and ovoid, singly or in chains, 3–9 μ in diameter. Elongated, branching filaments 2–4 μ in diameter. Subclavate cells terminally on filaments, 9–15 μ in long axis.

Yeast-Dextrose Agar (Difco, pH 7.0).—Macroscopic appearance similar to that on nutrient agar. Yeast-like budding cells 3–12 μ in diameter. Filaments monilioid, approximately 3 μ in diameter, branching, terminating in a double-contoured ovoid cell 12 μ in long axis; blastospores of varying proportions.

Nutrient Blood Agar (nutrient agar plus bacto-beef blood, pH 7.2).—Colony approximately 2 cm. in diameter with an irregular periphery. Yeast-like cells double-contoured, mostly spherical, in general 9 μ in diameter. Many smaller cells. Filaments 2–4 μ in diameter, cross-walled and branching.

Serum (Bacto-beef blood serum, pH 7.3).—Thick sediment at bottom of flask. Mycelium of filaments approximately 3 μ in diameter, terminating in a thick-walled, spherical, ovoid or sublanceolate cell 9–15 μ in diameter; blastospores in varying proportions.

Endo's Agar (Difco, pH 7.5).—Colony approximately 3 cm. in diameter. Mycelium mostly submerged in agar. Many yeast-like, budding cells 3–12 μ in diameter. Hyphae 2–4 μ in diameter. Blastospores spherical, ovoid or pyriform, approximately 6 μ in diameter or long axis.

Carbohydrate reactions.—Acid and production of gas with dextrose, maltose, levulose, and d-mannose. Acid and no gas with galactose, lactose, saccharose. No acid or gas with l-arabinose, l-xylose, rhamnose, raffinose, mannitol, amygdalin or dextrin.

Gelatine.—Gelatine is liquefied.

Litmus milk.—Shows no acid or curdling.

Cytology

As far as can be determined by the literature, there has been very little work on the cytology of the imperfect yeasts. Rajat ('06) described briefly a species of *Parendomyces* (?) under the name "champignon du muguet." The nuclear phenomena are often important in classification and in questions of phylogeny. The single yeast-like cell is uninucleate (pl. 15, figs. 27–30). The granular nucleus is seen as a large body with a heavily staining nucleolus from which emanates a net-work along which are granules of volutin deeply stained with haematoxylin. As the cell grows older, it elongates (pl. 16, fig. 31) and the cell wall is constricted. The nuclear division which then occurs at the point of indentation is amitotic, of an advanced type. No chromosomes are seen in the division process; instead, as far as could be determined from fixed material, there is a clumping of the metachromatic material in two groups, within the nucleus and opposite to each other (figs.

31–32, 35–36, 38, 42, 50). At this time, within the nucleus, fine reticulations with small granules extend between the two clumps. Directly after division these reticulations seem to spread out (figs. 33, 37, 50) and retain the type of structure seen in the large nuclei (figs. 27, 49).

When the young yeast-like cell or blastospores are allowed to germinate (pl. 15, figs. 1–4), the nucleus elongates, divides within the cell, and the daughter nucleus grows along with the tube as it grows out. In the formation of the cross-wall the daughter nucleus also elongates and divides amitotically as described above, accompanied by an abscission of the cell. One nucleus is thus carried into the newly formed cell. At a later stage, the mother cell divides in two, with one nucleus in each daughter cell, resulting in a uninucleate cell (pl. 16, figs. 39, 46, 48). In many of the yeast-like cells, the nucleus is at the apical portion growing into the newly forming bud (figs. 41–42). As the bud matures, there is an abscission from the mother cell and the nucleus is divided in the process (fig. 43).

The mycelium is uninucleate, with the extended nucleus centrally placed in the long cells (figs. 47–48). It is of interest that the division of a nucleus does not necessarily imply that a new cell is cut off at the same time. A nucleus may divide within a cell and then migrate into a newly formed bud (figs. 33, 36–37, 48–50). This is particularly true in the formation of the blastospores where the nucleus first divides amitotically and then migrates into the young blastospore which consequently is uninucleate.

The presence of a network and heavily granulated protoplasm is noted in all the cells. Except for the blastospores and the clavate, obovate or lanceolate cells which are modifications of the yeast-like cells, there are no distinctive structures in this organism.

Cellular contents.—Reserve materials are found normally in larger amounts in the older portions of the mycelium, as glycogen, lipoids, oil globules, metachromatic granules, or volutin, or as decomposition products, as nucleic acid substances, protein derivatives, or carbohydrates. These ma-

terials have been discussed previously in greater detail (Rajat '06; Moore '33, '35).

Volutin.—Volutin or metachromatic material may be very easily demonstrated with methylene blue (fig. 54) or even with iron-alum haematoxylin, as substances within the cell, along the inner wall surface, or along the network, both within the nucleus and in the cytoplasm. With the latter dye they stain deeply, whereas with the former they appear as droplets. A pinch of benzidine sulphate added to a water mount of living organisms (fig. 52) reveals a vacuolar condition within which are a varying number of granules known as "dancing bodies." These granules are precipitated volutin in a state of Brownian movement. They take a blue coloration supposedly, because of the action of benzidine on the peroxidases.

Glycogen.—With saturated iodine potassium iodide glycogen can be demonstrated very easily (fig. 55), taking an orange-brown coloration. With neutral red it is not quite so clear but appears as a pink or red vacuole called the glycogen vacuole. Glycogen is more abundant in the older than in the younger cells.

Vacuoles.—Vacuoles are easily demonstrated with benzidine sulphate (fig. 52), methylene blue (fig. 54), and with iron-alum haematoxylin. In the last case they appear surrounded by the network. Vacuoles are further brought out with iodine, within which are found the crystalloid droplets comparable to those seen with neutral red. They can also be demonstrated with saturated iodine potassium iodide.

Chondriosomes.—Chondriosomes are shown with saturated iodine potassium iodide, as demonstrated by Guilliermond. In this fungus they appear as light yellow, refractile bodies or droplets of varying size, distributed within cytoplasmic substance. There are few in the young cells.

Fat, lipoidal substances.—In addition to the substances mentioned, there are also fats, lipoidal substances, other reserve materials, and secretion and even excretion products. What

the nature of these substances may be is not entirely clear, but it is known that fatty acids, glycerides, phospholipides, glycerol and phospho-aminolipides (complex lipides) may be present in varying amounts. Several agents were tried to demonstrate them, each showing some advantage. With 2 per cent osmic acid, they are reduced and take a black coloration (fig. 51). In the young cells or filaments, the blackened portions are more abundant at points where the nuclei would be found. Many of the young, yeast-like cells show only a single small droplet, while older cells show numerous irregular globules throughout. With platinic chloride (5 per cent solution) much the same results are obtained (fig. 53). Iodine potassium iodide as applied for glycogen or chondriosomes shows lipoidal substances, as oil droplets, equally as well. These are very small and appear as refractile, hyaline bodies.

Systematic Position

Since the literature dealing with the nomenclature of the imperfect yeasts is voluminous and has been thoroughly summarized in a recent work by the senior author ('35), it need not be reviewed here. *Syringospora* Quinquaud ('68) was based on *S. Robinii,* a renaming of *Oidium albicans* Robin ('53), which in turn was based on the work of Gruby (1842) on the common organism of thrush (muguet, Soor or sapinho). The morphology figured by Quinquaud is similar to that of our species or to that of *Mycotorula* Langeron & Talice ('32) non Will. In our organism, the verticils show some reduction, being less dense than in *S. albicans* and tending to produce one or two branched chains of blastospores at the septa as in the genus *Mycotoruloides.* The verticils of these chains of blastospores are also reduced, approaching a state shown in *Mycocandida* where the verticils normally contain only two blastospores per septum of the axial filament. The blastospores are generally ellipsoidal, rarely clavate or obovate, never pyriform or lacrimiform as those of *Blastodendrion.* Thick-walled cells (perhaps chlamydospores) appear to be basal either to a whole pseudo-mycelium or to a primary branch thereof. They are

rarely terminal especially on serum (fig. 14 or 21), similar to those figured by Roger ('96) reproduced by Noisette ('98) for their strains of *S. albicans*.

In cultures our organism differs from *S. albicans* and *S. Braulti* in not forming a pellicle on any liquid media, although it is somewhat closer to the latter in morphology. Mazza & Palamedi report that sugars were not fermented nor gelatine liquefied, while Talice & Mackinnon ('34) and ourselves have found the organism to ferment glucose, fructose, mannose, and maltose, and to liquefy gelatine. It is possible that Mazza & Palamedi did not keep their cultures long enough to observe fermentation or liquefaction. After a study of Mazza & Palamedi's cultures, Talice & Mackinnon have reduced this species to synonymy with *Syringospora albicans* under the synonym *Mycotorula albicans* (Robin) Langeron & Talice. While this organism shows many characters in common with *S. albicans*, its invasive power is so much greater (being severely pathogenic to rabbit after two years in culture) that it hardly seems likely that it is the same as *S. albicans* which is a mild parasite of the mucosa of infants, or of extremely senile or moribund adults, having little or no power to invade the skin or lungs of adults. *S. inexorabilis*, starting in the lungs or buccal mucosa of a healthy farm laborer, produced a generalized infection involving the skin and invading the tissues.

While recognizing that only a thorough monograph along the lines of that undertaken by the Centraalbureau voor Schimmelcultures, but upon a variety of suitable media not used there, can settle the systematic position of these organisms, it seems better to transfer the species to *Syringospora*, where it may be characterized as follows:

SYRINGOSPORA INEXORABILIS (Mazza & Palamedi) Dodge, Med. Myc. 242. 1935.

Monilia inexorabilis Mazza & Palamedi, Reunión Soc. Argentina Patol. Reg. del Norte en Tucumán **7**: 424–467, *1 pl. 50 fig.* 1932.

Mycotorula albicans Talice & Mackinnon, Reunión Soc. Argentina Patol. Reg. del Norte, Santiago del Estero **8**: 165–166. 1934.

Isolated from blastomycosis of the skin and mucosa of a patient in the Argentine Chaco by Mazza and Palamedi ('32); pathogenic to guinea pig and rabbit. Perhaps the case of Rockwood & Greenwood ('34) should be referred here, although the autopsy findings were less positive.

Yeast cells in tissue and pus, spherical. Yeast cells common in media of low hydrogen-ion concentration and higher oxygen tension, thin-walled except on serum. In higher hydrogen-ion concentrations pseudo-mycelium abundant in favorable media, true mycelium or racquet mycelium in unfavorable media. Blastospores single or in short, branched chains, usually at the septa, occasionally scattered in poor media.

Related Species

Recently, Ciferri and Redaelli ('35) published notes on the morphology of several species of *Syringospora* and related genera, which had previously been very imperfectly known. As this information was received too late to incorporate in Dodge's 'Medical Mycology' ('35), it seems desirable to summarize it here and record the new combinations made necessary by the new information.

Syringospora **dimorpha** (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula dimorpha Redaelli & Ciferri, Arch. Mikrobiol. **6**: 43–46, *fig. 28, 29.* 1935.

Mycotorula interdigitalis Redaelli, 1930. non Pollacci & Nannizzi.

Isolated from dysidrosiform lesions of the interdigital spaces of a patient in Cairo, Egypt. (Soliman, strain 3). Pathogenic for guinea pig.

Hyphae long, septate, little-branched, producing dense verticils of blastospores at the septa. Blastospores $3 \times 3.5\ \mu$.

On glucose agar, colony rounded (3 cm. in 20 days), dense, creamy, dirty white tending toward yellowish, central portion somewhat elevated, broadly crateriform, smooth, with outer 6 mm. next margin grayish, deeply radially furrowed, margin round. On liquid media producing a ring on malt extract,

otherwise with a few floating islets which settle as an abundant floccose sediment. Fermentation of glucose, fructose and mannose, acid with the other carbohydrates. No action on milk; gelatin liquefied within 5 days.

MYCOTORULOIDES **macedoniensis** (Castellani) Dodge & Moore, n. comb.

Monilia macedoniensis Castellani & Chalmers, Man. Trop. Med. ed. 3, 1087. 1919.

Myceloblastanon macedoniense Ota, Jap. Jour. Derm. Urol. **28**: 127. 1928.

Castellania macedoniensis Dodge, Med. Myc. 259. 1935.

Mycotorula macedoniensis Redaelli & Ciferri in Ciferri & Redaelli, Arch. Mikrobiol. **6**: 18. 1935.

Originally isolated from sputum of a patient in Macedonia by Castellani. The culture studied by Ciferri and Redaelli was received from Castellani under the name *Monilia macedoniensis* var. *macedoniensoides* Cast., isolated from sputum. Castellani & Taylor ('25) originally described *Monilia macedoniensoides* in a paper on vaginal monilias, and while they do not specifically state that this species came from the vagina one would assume that such is the case. Later the same year, Castellani, Douglas & Thompson ('25) list it in a paper on infections of the bronchi without giving data as to its origin. Ashford ('31) studied two strains of this species from Castellani, reporting glucose and fructose fermented by both strains; maltose, sucrose, galactose, and inulin fermented by one strain which fermented maltose only once in nine sowings. Acid was produced with glucose, maltose, galactose, xylose; about half the time with lactose and raffinose; and about one-fourth the time with mannitol and dextrin.

On liquid media only the yeast stage of ovoid cells 3–4 × 4.5–5 μ, budding monopolar, rarely bipolar, rarely in short chains but no true filaments. On solid media (Difco nutrient agar with glucose) hyphae with verticils of blastospores at the septa. From Ciferri and Redaelli's illustrations, hyphae rarely branched, blastospores usually in compact verticils, occasionally in short, branched chains characteristic of *Myco-*

toruloides Lang. & Tal. Blastospores evidently variable, but no dimensions or magnifications of figures given.

Giant colony after 15 days in Difco nutrient agar with glucose at room temperatures, circular, 1 cm. in diameter, creamy white, smooth, shining, center slightly elevated, smooth, sloping gradually to the periphery with rare radial folds, margin smooth, thin, uniform or finally mammillate. Sanfilippo ('24), who studied Castellani's culture, reports sediment in broth; no turbidity nor pellicle.

Fermentation and acid production in glucose, fructose, galactose, sucrose, and inulin; milk coagulated; gelatin and serum not liquefied.

From both its morphology and biochemical characters this species seems to belong to *Mycotoruloides,* although the younger hyphae are suggestive of *Syringospora.* Var. *macedoniensoides* differs only in not coagulating milk.

MYCOTORULOIDES **trimorpha** (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula trimorpha Redaelli & Ciferri, Arch. Mikrobiol. 6: 35. 1935.

Candida insolita Redaelli in Graziano, Giorn. Batt. Immun. 5: 1070–1075. *1 fig.* 1933. non (Cast.) Basgal, Contr. Estudo Blastomycoses Pulmonares, p. 49. 1931.

Isolated from feces in cases of enteritis of infants by Graziano ('33). Not pathogenic for guinea pig.

In solid media only yeast cells present. In liquid cultures, hyphae long, little-branched, of long cells bearing dense verticils at the septa, blastospores of the verticils in short-branched chains, 2.4 μ in diameter with a large hyaline cell (chlamydospore) at the tip of the hyphae up to 10 μ in diameter. Under some conditions these may proliferate to 8–10 cells in a chain, suggesting the condition found in *Monilia.*

On Sabouraud, carrot, and malt-extract agars, colony smooth, white, creamy, margin smooth, surface shining; on malt, carrot and potato, medium colored yellow. On gelatin colonies color similar but opaque, penetrating somewhat into the medium. In carrot decoction and malt extract, complete

ring and incomplete thin pellicle, abundant sediment, and slight turbidity.

Obligate aerobe, optimum temperature 37° C., growth good but much slower at room temperature. Ferments glucose, fructose, maltose, galactose, and sucrose; slight acid production on most media; milk not coagulated nor digested; gelatin and serum not liquefied.

PARENDOMYCES **Flareri** (Redaelli & Ciferri) Dodge & Moore, n. comb.

Blastodendrion Flareri Redaelli & Ciferri, Arch. Mikrobiol. 6: 51–53, *fig. 35, 36.* 1935.

Strain 2 Cazzani was isolated by Flarer from scaling erythematous lesion of the human skin, and Strain 8 from an eczematous lesion, both in Messina, Italy. Not found pathogenic for guinea pig by intravenous injection.

Cells sprouting, forming small branched groups, rarely short, highly branched filaments, of blastospores variable in shape and size, 1.5–2.3 × 2–3 μ; chlamydospores 5 μ in diameter; cells filled with dense homogeneous protoplasm or uniguttulate except in old vacuolate cells.

On Sabouraud agar, colony round, dense, creamy, white, center elevated, slightly crateriform, sloping gently to the smooth margin, surface rather dull. In liquid media only an incomplete ring, with discrete sediment of conglutinate floccose colonies; in some media with a diffuse opalescence. No fermentation of carbohydrates; acid in dextrose, levulose, and mannose; no action on milk, nor on gelatine. Strain 8 also produced acid in galactose and raffinose.

Apparently differs from *P. Perryi* (Castellani) Dodge, in not producing acid on maltose.

PSEUDOMONILIA **verticillata** (Redaelli & Ciferri) Dodge & Moore, n. comb.

Mycotorula verticillata Redaelli & Ciferri, Arch. Mikrobiol. 6: 40. 1935.

Isolated at Milan, Italy, from a scaling erythematous lesion of the skin. Pathogenic for guinea pig.

Hyphae branched with verticils at the septa, verticils often of only two cells. Occasionally tufts of mycelium rise above the colony when the verticils are denser, suggesting *Syringospora;* blastospores ovoid to spherical, about 3.5 μ in diameter.

On glucose agar, colony irregularly rounded, much elevated above the substrate (10–12 mm. in diameter in 20 days at room temperature), shining, whitish, opaque, cerebriform, irregular, marginal lobes smooth and thick. On liquid media, a ring with confluent islets forming a pellicle and abundant sediment. No fermentation of sugars; acid in glucose, mannose, fructose, galactose, and maltose; no action on milk or gelatin.

From its biochemical characters this species seems to belong rather to *Pseudomonilia* than to *Syringospora* (*Mycotorula* Lang. & Tal.). Morphologically the aerial mycelium sometimes suggests the latter, although in liquid media and within the colony it is typical of *Pseudomonilia.*

Pseudomonilia **zeylanoides** (Shaw) Dodge & Moore, n. comb.

Monilia zeylanoides Shaw, Centralbl. f. Bakt. I. **119**: 460–464. 1931. non Cast.

Mycotorula zeylanoides Redaelli & Ciferri, Arch. Mikrobiol. **6**: 41–43, *fig. 26, 27.* 1935.

Isolated by Shaw from sputum and referred to *Monilia zeylanoides* Cast in 1931. Isolated by Carco from tonsillar lesions with the usual symptoms of a mycosis.

Hyphae long, hyaline, septate, with moderate branching, blastospores in reduced verticils at the septa.

On solid media colony dense, yellowish-white, becoming intense yellow in age, surface shining, margin smooth at first, becoming fringed. Giant colony showing three zones, center somewhat elevated and crateriform, second irregularly hilly suggesting lava flows, dense, milk-white, outer zone creamy color, thinner, opaque, finely fringed. On liquid media, complete ring, tough, dense, white, also thin pellicle of coalescing islets with abundant sediment. No fermentation of sugars; acid in glucose, fructose, mannose, galactose, maltose, inulin, and xylose; no action on milk; gelatin not liquefied.

This species differs from *Parendomyces zeylanoides* (Cast.) Dodge, to which it was referred by Shaw, in not coagulating milk, and in its abundant hyphae. It seems more closely related to *Pseudomonilia matalensis* (Cast.) Dodge, from which it differs in the thinner white pellicle and abundant sediment in liquid media, and the production of acid in most sugars, acid production in *P. matalensis* being slight or none.

MYCODERMA **desidiosum** (Ciferri & Redaelli) Dodge & Moore, n. comb.

Candida desidiosa Ciferri & Redaelli, Arch. Mikrobiol. **6**: 62–64. 1935.

Isolated from the intestinal contents of pigeons with experimental beriberi along with *Candida Krusei*. Pathogenic for guinea pigs.

Mycelium of short ellipsoidal blastospores, somewhat branched, cells filled with oil droplets.

On glucose agar, colony round, 1.5 cm. in diameter, cream to yellowish, dense, shining, central portion irregularly rounded, elevated but flat, smooth, with a few slight hillocks at the center; outer slope steep to margin which is smooth or slightly and indistinctly mammillate. In age, a slight grayish halo with a finely plumose margin so that three zones appear; a central plateau, an irregular mammillate, smooth, shining white zone, and a very thin grayish plumose margin. On liquid media, ring incomplete, better developed and yellowish on malt extract, slight turbidity, sediment in a single conglutinate mass. No fermentation.

CANDIDA **dendritica** (Ciferri & Redaelli) Dodge & Moore, n. comb.

Trichosporon (Geotrichoides) dendriticum Ciferri & Redaelli, Arch. Mikrobiol. **6**: 53–58, *fig. 37–38*. 1935.

Isolated along with *Blastodendrion Pinoyi* (Cast.) Lang & Tal. from a patient with bronchomoniliasis in Milan, Italy. Pathogenicity not stated.

Mycelium 1.5–2.3 μ in diameter, somewhat verticillately branched. Arthrospores abundant, 2.3–2.5 × 7–9 μ; blasto-

spores rare, ellipsoidal, tending to occur in verticils as in *Syringospora*.

On glucose agar, central portion whitish, creamy, mycelium mostly submerged in dendritic fascicles, with a thin, branched plumose margin. In liquid media a thin pellicle within 24 hours. Glucose and fructose fermented.

This species has been too imperfectly described for certain systematic position, but since *Trichosporon* is held to be untenable as a generic name, it seems best to place it here, pending further study.

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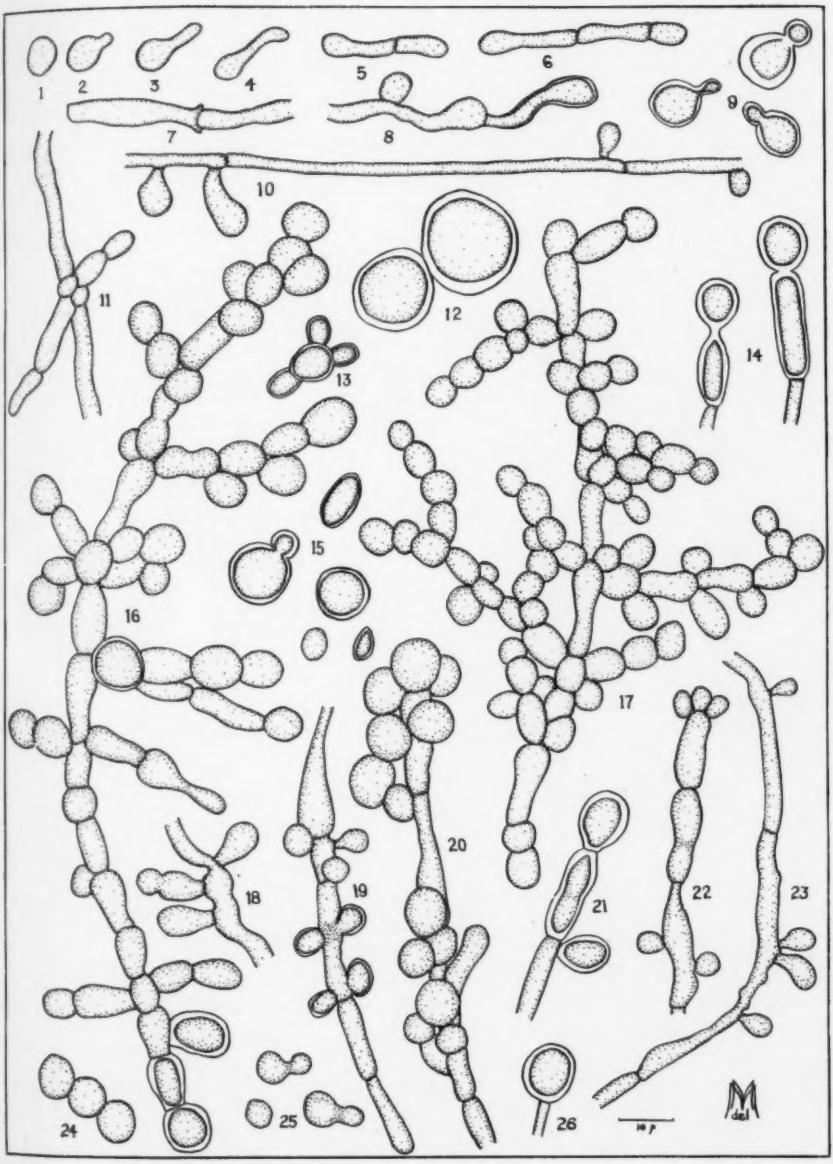
Explanation of Plate

PLATE 15

Syringospora inexorabilis (Monilia inexorabilis)

All figures drawn with the aid of a camera lucida at a magnification of × 1440 and reduced to × 765.

Figs. 1–6. Cells developing from blastospore or yeast-like cell.
Fig. 7. Collar-like cross-wall on Endo's agar.
Fig. 8. Type of mycelium on Czapek's agar.
Fig. 9. Group of budding yeast-like cells on malt extract agar.
Fig. 10. Type of mycelium with blastospores, on lactose agar.
Fig. 11. Type of mycelium on yeast-dextrose agar.
Fig. 12. Giant, spherical thick-walled cells on wort agar.
Fig. 13. Cells on wort agar.
Fig. 14. Type of cells in serum.
Fig. 15. Cells on yeast-dextrose agar.
Figs. 16–17. Type of mycelium on Raulin's solution.
Fig. 18. Blastospores on Czapek's agar.
Fig. 19. Mycelium with blastospores on Endo's agar.
Fig. 20. Mycelium with blastospores on nutrient blood agar.
Fig. 21. Cells in serum.
Figs. 22–23. Mycelium on Endo's agar with peculiar oidioid cells at apex (fig. 22).
Figs. 24–25. Cells on nutrient blood agar.
Fig. 26. Cells in serum.



DODGE & MOORE — SYRINGOSPORA INEXORABILIS

Explanation of Plate

PLATE 16

Syringospora inexorabilis (Monilia inexorabilis)

All figures drawn with the aid of a camera lucida. Figs. 27–50 drawn at a magnification of × 2300 and reduced to × 1220. Figs. 51–55 drawn at a magnification of × 1440 and reduced to × 765.

Figs. 27–29. Uninucleate cells showing metachromatic material in cytoplasm and nucleoplasm.

Fig. 30. Cell showing elongation and early contraction of walls.

Fig. 31. Elongated cell showing early concentration of nucleoplasmic granules at opposite ends of nucleus.

Fig. 32. Early stage in division of nucleus with concentration of metachromatic material.

Fig. 33. Showing a divided nucleus and an elongated nucleus ready for division.

Fig. 34. Nucleus prior to concentration of metachromatic material.

Fig. 35. Nucleus which is to divide by transverse abscission of cell.

Fig. 36. Budding cell with dividing nucleus.

Fig. 37. Budding cell with divided nucleus. A daughter nucleus will migrate into the bud.

Fig. 38. Apical cell showing transverse abscission and division of nucleus.

Fig. 39. Cell ready to divide, with nuclei at opposite ends.

Fig. 40. Large budding yeast-like cell prior to division of nucleus.

Figs. 41–42. Budding yeast-like cells with nuclei pushing into buds.

Fig. 43. Yeast-like cell which has budded and divided the nucleus amitotically.

Fig. 44. Early stage in transverse abscission of cell and nucleus.

Fig. 45. Group of cells showing nuclei at point of contact.

Fig. 46. Group of cells ready to divide as fig. 39.

Fig. 47. Elongated, narrow hyphae showing blastospores and centrally located, elongated nuclei in filament.

Figs. 48–50. Mycelium showing budding blastospores and dividing nuclei in various stages of development.

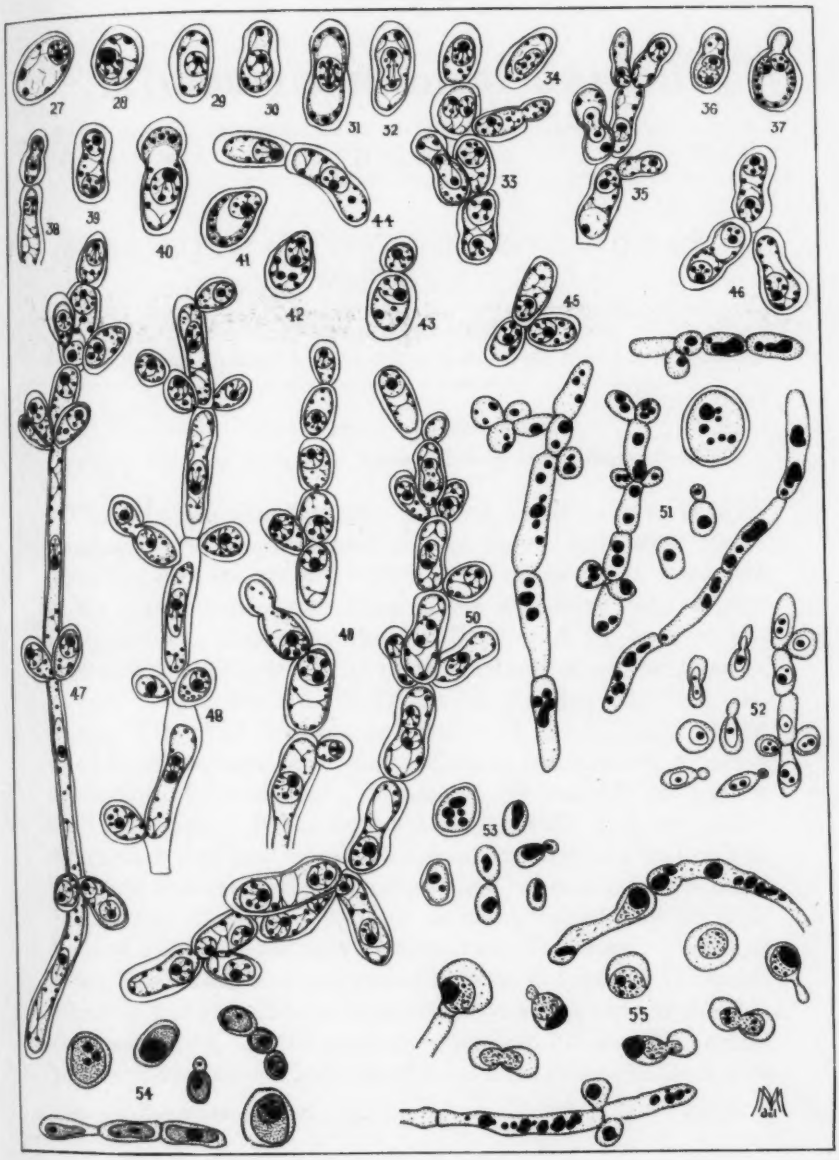
Fig. 51. Mycelium mounted in 2 per cent osmic acid, showing fat globules.

Fig. 52. Mycelium mounted in distilled water with a pinch of benzidine sulphate, showing vacuoles and "dancing bodies."

Fig. 53. Mycelium mounted in 5 per cent platinic chloride, showing the same picture as with osmic acid.

Fig. 54. Mycelium mounted in 1 per cent aqueous methylene blue, showing precipitated volutin.

Fig. 55. Mycelium mounted in saturated iodine potassium iodide, showing glycogen as dark globules, fat and lipoidal substances as small refractile bodies, and possible chondriosomes as short rods.



DODGE & MOORE — SYRINGOSPORA INEXORABILIS